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[1] Numbered 68 in text.
[2] Numbered 69 in text.

ERRATA

VOLUME II

Page 375, line 5 from foot of page, "Sörmer" should read "Störmer."

VOLUME III

Page 8, reference (3), "t. 4" should read "t. 74."

Page 124, Table VIII, heading "Strength of solution" should be in top line of table with "Tops" and "Roots."

Page 130, Table XIV, top line, second and third rules should be moved one column to the left; fourth rule should not project into this line.

Page 135, lines 6-7, omit "following."

Page 135, line 7, insert "in Table XIX" after "results."

Page 135, line 20 from foot of page, "greater if the pressure is not so great" should read "less when the pressure was not so great."

Page 289, title of article, omit second "the."

Page 431, footnote, after "gl sal" insert "grn—granule."

Page 446, line 2, "on" should read "of."

Page 453, line 12 from foot of page, "palliated" should read "papillated."

Page 454, right hand column, after "recessus," "dentatus" and decurrens," add "n. sp."

Page 486, "Fig. 68" should read "Fig. 69" and "Fig. 69" should read "Fig. 70."

Page 500, legend of figure 1, line 2, "halve" should read "halves."

SOIL SCIENCE
RUTGERS COLLEGE

VOL. III NEW BRUNSWICK, N. J., JANUARY, 1917 No. 1

THE ORGANIC MATTER OF THE SOIL: III. ON THE PRODUCTION OF HUMUS FROM MANURES[1]

BY

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INTRODUCTION

In 1872 Grandeau (3) devised a method for the estimation of the *Matière noire* of soils by first leaching the soil with dilute acid to set the humus compounds free from their combination with the alkaline earths and then dissolving out the humus, or *matière noire,* with ammonia. He believed that the organic matter which dissolved was responsible for the fertility of the soil, apparently not so much because of the carbon or nitrogen which it contains as for the high percentages of phosphoric acid and potash in the humus ash.

The opinions of Grandeau have not been generally adopted in Europe but, until recently, they have found a wide following in America, due probably to the whole-hearted acceptance of Grandeau's humus theory by the late Professor Hilgard.

Hilgard (4, Chap. VIII) believed that the organic matter which has become an integral part of the soil must first be converted by humifying bacteria and fungi into humus before the nitrogen which it contains can become available to the nitrifying bacteria, even going so far as to say (4, p. 360) *"as a current source of nitrogen to the plant the unhumified matter is hardly entitled to more consideration than the insoluble silicates."*

The idea that the soluble humus of a soil is an indication of the potential fertility of that soil and that the humus nitrogen plays an all important rôle in the nutrition of plants, has been recently called in ques-

[1] The work embodied in this paper was carried out while the author, now Associate Agricultural Bio-Chemist, was Associate Soil Chemist.

Received for publication October 2, 1916.

tion by Weir (8). Weir found that although he removed 40 per cent of the nitrogen by extracting the humus from the soil with sodium hydroxide, when the extracted soil was subsequently used for vegetative experiments "approximately equal total yields both of dry matter and of nitrogen were obtained over four successive crops. It thus appears that the removal of the soluble humus had no effect in diminishing the productiveness of the soil in spite of the fact that the soil used was known to respond to nitrogenous fertilizers." Weir found that the removal of the soluble humus caused an increased ammonia production and a decreased nitrate production in the soil, the sum of the ammonia and nitrate being usually less in the treated soil than in the untreated soil.

In justice to the earlier work it must be pointed out that in all probability a very considerable portion of the humus nitrogen still remained in Weir's extracted soil, inasmuch as the writer (1) has shown that by a single extraction with 4 per cent NaOH it is possible to remove from 61.2 to 74.5 per cent of the total soil nitrogen, or an average of 68.5 per cent for eight mineral soils. Using repeated extractions, made in much the same manner as Weir describes, the author (2) was able to remove 81.9 per cent of the soil nitrogen by leaching with acid and nine subsequent extractions with 4 per cent NaOH, (N content; original soil 0.908 per cent; extracted soil 0.164 per cent) and in subsequent extractions with water, a soil residue containing only 0.088 per cent of nitrogen, or a decrease of 90.3 per cent from the original nitrogen content of the soil, was obtained. This would indicate that almost all of the soil nitrogen may be extracted with sodium hydroxide.

In the preceding paper (1), the writer has made a study of the solubility of nitrogen and carbon in soils as compared with unchanged vegetable materials and peats, and found that it is extremely doubtful that a specific "humification" of plant materials takes place in the soil, giving rise to an increased amount of ammonia-soluble humus. Snyder (6, 7) in 1897 prepared artificial humus by mixing a subsoil with certain organic substances and allowing these to remain in a moist condition for one year. At the end of the year humus was determined on the resulting mixture by extraction with ammonia following a previous leaching with dilute acid, and the humus so found was regarded as having been formed in the soil during the preceding year. It is unfortunate that Snyder neglected to determine the amount of ammonia-soluble materials present in the mixtures *at the beginning of the experiment.* The soil used contained only 0.06 per cent of humus but as the writer has already shown (1) ammonia dissolves a very considerable portion of material from unchanged organic compounds so that the "humus" gain at the end of the experiment may actually have been a loss when compared with the amount of ammonia-soluble materials at the beginning of the experiment. That it actually was a loss is demonstrated by the work which follows.

A series of experiments were carried out on the same general plan as was adopted by Snyder, with the exception that the ammonia-soluble materials were determined both at the beginning and at the end of the experiment, and in each instance there was a *loss* of ammonia-soluble material.

EXPERIMENTAL

Earthenware jars, each provided with a hole in the base for drainage, were filled with a mixture of subsoil and organic materials as follows[1]:

Jar A. 7500 gm. of moist subsoil and 500 gm. of a mixture of silk waste and quartz which had been ground to an impalpable powder in a steel ball mill. The mixture had a nitrogen content of 4.50 per cent, indicating that about 140 gm. of protein had been added.

Jar B. 7500 gm. of moist subsoil and 460 gm. of a mixture of one part cleaned wool and two parts quartz which had been ground to a powder in a steel ball mill. The nitrogen content of the wool and quartz mixture was 4.53 per cent.

Jar C. 7500 gm. of moist subsoil, 50 gm. of powdered calcium carconate and 300 gm. of a high grade "patent" flour.

Jar D. 7500 gm. of moist subsoil and 200 gm. of alfalfa meal. Analyses of this alfalfa meal have been reported in connection with one of the preceding papers (1).

The subsoil used was from a third foot bulk sample taken from a grove on the farm of the Minnesota Agricultural Experiment Station. The moist soil contained 13.4 per cent of water, thus reducing the 7500 gm. of soil taken to 6495 gm. of oven-dry soil.

After mixing the organic substances with the soil a sample of about one pint was taken, spread out on a paper in the greenhouse until air dry, bottled and set aside for analysis. The remaining soil was placed in the earthenware jars and moistened with water containing approximately 50 c.c. of an almost clear suspension of soil bacteria.[2] All of the jars were then planted to barley but inasmuch as poor growth was obtained on some of the jars, the vegetation test was soon discontinued, the barley stalks cut off and the jars of soil allowed to remain in the greenhouse under fallow conditions, care being taken to keep the soil moist.

At the end of one year samples were taken from each jar representing depths of 0"-3" and 4"-6", the remainder of the soil being left in the jar for further action by bacteria and fungi. The samples which were removed were dried at 65° C., ground to pass a 1-mm. sieve and bottled for analysis.

[1] A number of other jars were prepared, seventeen in all, but inasmuch as these have not been analyzed, it is useless to give the details of their preparation.

[2] Prepared by stirring up with water a sample of soil of 0-6" depth from an alfalfa field, allowing the soil to settle and decanting the clear supernatant liquid.

During the course of the experiment some changes were observed in Jars A and B. Fourteen days after the experiments were started black streaks were apparent through the soil in Jar A about 4 to 5 mm. below the surface. In Jar B there was a layer of black soil 3 to 4 mm. thick and about 5 mm. below the surface. Below this the soil was again the original yellow color. The location of the black layer was probably determined by aeration and moisture conditions. In some lumps lying upon the surface the entire center of the lump, in some instances as large as a walnut, was decidedly black.

One year after the experiment was started the samples were removed for analysis. No noteworthy changes in coloration could be observed in either of Jars C or D. In Jar A purple spots appeared throughout the soil. These spots were in some instances 5 cm. in diameter and occasionally ran into each other so that the soil was more or less completely filled with purple-red spots. The soil had a very mouldy odor and mould mycelia were easily observed. Jar B was similar to Jar A with the exception that the spots were black instead of purple. The 0″-3″ section was very spotted while the 4″-6″ section was uniformly dark, being, when moist, almost as black as a good surface soil. Here again the mouldy odor was very pronounced but without other data it is impossible to say whether or not the color was caused by bacteria or fungi. The soil from Jars C and D had in each instance a mouldy odor but had no black appearance, so that the presence of mould mycelia cannot be definitely taken as the causation of the black or purple colors.

The analytical data. The samples of soil were all analyzed when air dry and all results then calculated to the oven dry basis. Nitrogen was determined by Kjeldahling, 35 c.c. of concentrated H_2SO_4, 10 gm. of K_2SO_4 and a crystal of $CuSO_4$ being used. Carbon was determined by wet combustion in the apparatus recently described by the writer (1). Humus was determined both after leaching the soil with 1 per cent HCl and without such a previous leaching, by rotating 15 gm. of soil with 750 c.c. of 4 per cent NH_4OH in the apparatus as described previously (1), removing the suspended clay with ammonium carbonate, evaporating an aliquot of the liquid to dryness in quartz dishes, drying at 105° C., weighing, incinerating, and weighing again.

The "humus" solutions from the soils which had remained in the greenhouse for one year were but slightly darker than the extracts from the original mixture, and were in no instance black, being rather a very light brown.

The analytical data follow in Tables I, II and III. Table IV shows the differences between the analyses of the subsoil plus the unchanged materials added, and the subsequent analyses after the mixture had stood in a greenhouse under growing conditions for one year.

It will be observed that there is a loss in each instance of organic car-

bon, nitrogen and "humus from the leached soil." In two instances there is a slight gain (probably within experimental error) in the "humus from the unleached soil" and in the remaining two soils (A and D) there is a loss, of which that from D only appears to be significant.

TABLE I

ANALYTICAL DATA FOR ORGANIC CARBON AND NITROGEN ON THE DIFFERENT SOIL SAMPLES, TOGETHER WITH C/N RATIOS

Soil Number	Organic Carbon			Nitrogen			Carbonate CO_2	Ratio C/N
	I %	II %	Aver. %	I %	II %	Aver. %	%	
Original Subsoil.....	0.468	0.469	0.469	0.050	0.050	0.050	0.03	9.38
A-1915	1.310	1.330	1.320	0.331	0.336	0.334	0.03	3.95
A-1916—0"-3"	0.920	0.940	0.930	0.302	0.305	0.304	0.05	3.06
B-1915	1.460	1.470	1.460	0.348	0.350	0.349	0.04	4.18
B-1916—0"-3"	0.920	0.930	0.930	0.259	0.261	0.260	0.04	3.57
C-1915	2.090	2.150	2.120	0.118	0.119	0.119	0.25	17.81
C-1916—0"-3"	0.740	0.720	0.730	0.113	0.114	0.113	0.17	6.46
D-1915	1.820	1.780	1.800	0.150	0.152	0.151	0.06	11.92
D-1916—0"-3"	0.810	0.800	0.810	0.110	0.112	0.111	0.06	7.29

We have here no evidence of the formation of a specific "humus" but on the contrary there is a decided loss from this fraction. The percentage losses of nitrogen and "humus from the leached soil" are almost

TABLE II

ANALYTICAL DATA FOR HUMUS AND HUMUS ASH IN THE DIFFERENT SOIL SAMPLES, AFTER LEACHING WITH 1 PER CENT HCl

Soil Number	Humus			Humus Ash		
	I Per cent	II Per cent	Average Per cent	I Per cent	II Per cent	Average Per cent
Original Subsoil	0.57	0.59	0.58	0.43	0.41	0.42
A-1915	0.75	0.75	0.75	0.41	0.37	0.39
A-1916—0"-3"	0.69	0.65	0.67	0.24	0.28	0.26
B-1915	1.16	1.18	1.17	0.41	0.41	0.41
B-1916—0"-3"	0.85	0.81	0.83	0.31	0.31	0.31
C-1915	0.76	0.80	0.78	0.40	0.38	0.39
C-1916—0"-3"	0.73	0.73	0.73	0.37	0.36	0.36
D-1915	1.26	1.25	1.26	0.31	0.31	0.31
D-1916—0"-3"	0.85	0.83	0.84	0.32	0.32	0.32

identical and decidedly different from those of the organic carbon. It would appear possible that an ammonia extraction following a previous leaching with HCl dissolves mainly proteins, in which case the ammonia-soluble material and nitrogen would be causally related, although if this be the case it is difficult to see where the carbon losses of A and B originated, for the large carbon losses here could apparently come only

from protein material. Until much further work is done these relations must remain obscure.

It is of interest to observe that there is a much greater wastage of carbon than of nitrogen. Hilgard (4, p. 123-124) calls attention to the increased nitrogen content of the humus over that of the original vege-

TABLE III

ANALYTICAL DATA FOR HUMUS AND HUMUS ASH ON THE DIFFERENT SOIL SAMPLES, *WITHOUT* PREVIOUS LEACHING WITH 1 PER CENT HCl

Soil Number	Humus			Humus Ash		
	I Per cent	II Per cent	Average Per cent	I Per cent	II Per cent	Average Per cent
A-1915	0.59	0.61	0.60	0.31	0.27	0.29
A-1916—0″-3″	0.55	0.57	0.56	0.27	0.29	0.28
B-1915	1.04	1.06	1.05	0.37	0.37	0.37
B-1916—0″-3″	1.12	1.02	1.07	0.29	0.27	0.28
C-1915	0.73	0.76	0.75	0.27	0.28	0.28
C-1916—0″-3″	0.79	[1]1.14	0.79	0.25	0.25	0.25
D-1915	1.57	1.52	1.55	0.33	0.36	0.35
D-1916—0″-3″	0.61	0.61	0.61	0.29	0.27	0.28

[1] Obviously erroneous and therefore discarded from the average.

table materials. If we take the average carbon content of proteins as 51.15 per cent [average of 30 analyses given by Mathews (5, p. 110)] a C/N ratio of 3.06 found in soil A-1916 would give a nitrogen content of 16.71 per cent which approaches very nearly to the average nitrogen

TABLE IV

CHANGES OF CARBON, NITROGEN AND HUMUS IN THE EXPERIMENTAL SOILS DURING ONE YEAR'S EXPOSURE IN THE GREENHOUSE

Soil No.	Change of Organic Carbon 1915-1916		Change of Nitrogen 1915-1916		Change of Humus from Leached Soil 1915-1916		Change of Humus from Unleached Soil 1915-1916	
	% of Soil	% of Original Carbon	% of Soil	% of Original Nitrogen	% of Soil	% of Original Humus	% of Soil	% of Original Humus
A	—0.39	29.5	—0.030	9.0	—0.08	10.6	—0.04	6.6
B	—0.53	36.3	—0.089	25.5	—0.34	29.0	+0.02	1.9
C	—0.39	18.4	—0.006	5.0	—0.05	6.4	+0.04	5.3
D	—0.99	55.0	—0.040	26.5	—0.42	33.3	—0.94	60.6

content of these thirty proteins, i. e., 17.66 per cent. It is evident that the materials remaining in the soils are rapidly increasing in nitrogen content and further analyses at a later date should prove highly interesting.

SUMMARY

An attempt was made to see whether an increase of ammonia-soluble "humus" could be brought about by adding certain organic substances to a subsoil and allowing the mixture to undergo natural "humification."

Powdered silk waste, powdered wool, flour and alfalfa meal were the substances added to the subsoil. Carbon, nitrogen, and "humus" were determined on the original mixture and on the final product after remaining in a moist condition in the greenhouse for one year. The following conclusions are evident:

1. There is a decided loss of organic carbon from all of the samples ranging from a minimum loss of 18.4 per cent to a maximum of 55 per cent of the carbon originally present.

2. There is likewise a loss of nitrogen but this loss is not proportional to the loss of organic carbon ranging from 5.0 to 26.5 per cent of the nitrogen originally present.

3. *There is a loss of "humus,"* the material dissolved from the leached soil by 4 per cent NH_4OH, and this loss appears to be directly proportional to the loss of nitrogen. Whether there is a causal relationship here can be determined only by more extended work. The fact that earlier investigators obtained a *gain* in humus following a year's "humification" is readily explained by the fact that no allowance was made for the presence of ammonia-soluble substances originally present in the materials added to the subsoil.

4. In three of the four experiments no significant change is apparent in the materials extracted by 4 per cent NH_4OH from the unleached soil. In the remaining experiment there is a loss of 60 per cent of the original materials.

5. These experiments furnish no evidence that an increase of soil "humus" is brought about by a specific humification. On the contrary, all of the evidence is directly opposed to such a conclusion, and it appears altogether probable that the maximum amount of ammonia-soluble material is present in a soil immediately after a green manuring crop has been plowed under and before the "humifying" bacteria or fungi begin their work.

Addendum

January 10, 1917

Since this paper was submitted for publication, the attention of the author has been called to Bulletin 129 of the Texas Agricultural Experiment Station by G. S. Fraps and N. C. Hamner, entitled, "Studies of the Ammonia-Soluble Organic Matter of the Soil" published in June, 1910.

In Part II (pages 23-30) the authors conducted experiments similar to those which are reported in the present paper and arrive at the same conclusion that is given in No. 3 of the Summary above.

The author regrets exceedingly that this work was not given proper recognition. Mention of this bulletin should also have been made in the first paper of the series (1) inasmuch as Fraps and Hamner point out that "organic matter added to the soil already contains ammonia-soluble

material. When no correction is made for the ammonia-soluble substances in the added material, ammonia-soluble humus is apparently formed in the decay of cottonseed meal, etc., but when correction is made for the added ammonia-soluble material the ammonia-soluble material is found to decrease."

The present paper, however, covers not only the estimation of "humus," in which feature it merely repeats and confirms Fraps and Hamner's work, but it also includes in addition comparative analyses of organic carbon and of nitrogen.

The method of "humification" is likewise somewhat different, inasmuch as the present experiments were carried out in a greenhouse under normal conditions of light, warmth and moisture, while the experiments of Fraps and Hamner were conducted in quart jars, stored in a dark basement and kept at a moisture content equal to one-third of the saturation capacity of the soil. They apparently used a surface soil (original humus 1.29 per cent) mixed with the various organic materials. The conditions in the present experiments approximated more nearly those conditions under which a specific "humification" has been supposed to take place, while their conditions were more nearly those favoring "decay."

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THE LOESS SOILS OF THE NEBRASKA PORTION OF THE TRANSITION REGION: VI. THE RELATIVE "RAWNESS" OF THE SUBSOILS[1]

By

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INTRODUCTION

In the first paper of this series (4, p. 234) it was mentioned that we had found inoculated legumes to grow almost as well on the Nebraska loess subsoils from depths of 3 to 20 feet as on the corresponding black surface soils, but that on the same subsoils non-leguminous plants failed to make any satisfactory growth, unless treated with a nitrogen fertilizer or preceded by leguminous crops.

In the agricultural practices of humid regions the "rawness," or infertility, of the subsoil has long been recognized. In plowing, care is taken to avoid bringing up much of the subsoil. In leveling rough land the surface soil is carefully scraped off into a pile to be spread evenly over the surface after the grading has been completed; in digging a small pit, or even a post-hole, the same precaution is commonly observed. This generally recognized unproductivity of humid subsoils, when freshly brought to the surface is frequently contrasted (6, p. 164; 7, p. 102; 9, p. 28; 11, p. 162; 13, p. 29; 14, p. 82; 16, p. 527; 18, p. 15) with the lack of any such "rawness" in the subsoils of arid regions, to which the attention of investigators was first called by Hilgard[3] (10, p. 19-20) but which may well have been recognized since prehistoric times in the agricultural practices of arid regions.

The relatively low nitrogen content of the subsoils of humid regions may serve to explain largely the unproductivity of these in the case of non-leguminous plants, but their sterility toward inoculated legumes is to

[1] Received for publication November 10, 1916.

[2] The work reported in this paper was carried out at the Nebraska Agricultural Experiment Station in 1911-1913, where the authors were respectively Chemist, Research Assistant in Chemistry and Assistant in Chemistry.

[3] He mentions the following example: "In the case of a cellar 7 to 10 feet deep, near Nevada City, Cal., the red soil-mass dug out was spread over part of a vegetable garden close by, and as a venture the annual vegetables—tomatoes, beans, watermelons, etc.—were sown just as usual. They not only did well, but better than the portions not covered, which had been cultivated for a number of years and were somewhat exhausted thereby." (10, p. 20.)

be attributed to a lack of availability of the phosphoric acid or of the potash or of both. That the "rawness" of the humid subsoils toward legumes as well as non-legumes is generally assumed is evident from the references given above. Thus, referring to California, Wohltman, who had previously made two visits to that state (18, p. 6) remarked in 1904 (18, p. 15):

"When with us in Germany, the subsoil is thrown out of a ditch, in the first year usually *nothing*[1] grows on the edge of the ditch. In America one can throw up the subsoil from a depth of five to six meters and on this see new plantings, peach trees and grapes, thrive just as well as on the adjacent old surface soil."[2] In reporting on a third visit to California he again calls attention to this peculiarity of the arid soils (19, p. 9).

Hilgard has made it clear that in the case of arid subsoils "rawness" appears as little with non-legumes as with legumes, mentioning in one of his last (1912) articles (12, p. 418) that "such a heap now lying before my eyes,[3] the upper layer of which had eight months before been excavated from a depth of 4 meters and still retained the last rain, shows a thick stand of grasses, and weeds of all kinds, among them wild oats, radish and mustard. . . . . ."

Hilgard (10, p. 19-20) attributed the infertility of the subsoils of humid regions to the washing down of the finest soil particles from the surface layers and their accumulation in the upper portion of the subsoil. The resulting comparative impermeability of the latter causes an insufficient aeration, and a reduction in the number of beneficial microörganisms, and may lead to unfavorable reduction processes and the formation of toxic substances (6, p. 165).

Ehrenberg (6, p. 163-165) fully endorses Hilgard's explanation, analyzing in detail the contributory factors. Conditions which favor deflocculation of the soil colloids, especially the colloidal clay, hasten the downward transport of these and the formation of the dense, impervious, "raw" subsoil. When the water from rain and melted snow, in itself poor in electrolytes, comes into contact with the soil it is much more enriched with salts in arid than in humid regions, the soluble salt and the carbonate content of the former being in general much the higher. The rain water dissolves electrolytes from the soil more rapidly during the warmer portion of the year than when the temperature is but little above freezing point. Other conditions being similar the proportion of carbon dioxide in the soil atmosphere increases with rising temperature of the soil (20, p. 122) and this not only in itself acts to flocculate the colloidal clay but also determines the amount of calcium carbonate dissolved. The water that falls during the warm season is largely returned to the atmosphere by

[1] The italics are those of the authors and do not appear in the original.
[2] Translation by the authors.
[3] In Berkeley, California.

transpiration and direct evaporation before it has had time to penetrate any considerable distance into the subsoil and so before it has been able to cause any considerable movement of either the colloids and finest silt particles or of the soluble salts and carbonates which flocculate the colloids and so hinder their translocation.

Characteristics of the Loess Soils

The properties, chemical and physical, of the loess soils of the Nebraska portion of the Transition Region have been dealt with in the earlier articles of this series. Here we will allude to only those characteristics which may be expected to bear more or less directly upon the fertility of the subsoil compared with that of the surface soil.

The nitrogen content, in the case of virgin fields, we found to lie between 0.159 and 0.318 per cent for the surface 6-inch layer and between 0.025 and 0.054 per cent for the sixth foot (4, p. 220-223), the latter being thus approximately one-sixth as high as the former.

The potash (3, p. 301, 303 and 307), both the total amount and the portion soluble in strong hydrochloric acid, is quite similar in subsoil and surface soil; the proportion soluble in 1 per cent citric acid increases with the relative aridity, in the most humid areas decreasing from the surface downward, but in the distinctly semi-arid areas increasing, notwithstanding an accompanying increase in the carbonate content, which lessens the solvent action of the acid.

In the first two feet the total phosphoric acid (3, p. 302, 303-310), almost the whole of which is soluble in strong hydochloric acid, is quite similar in amount throughout the region. In the lower sections, 3 to 6 feet, of the eastern areas it is higher. The citric-acid-soluble portion, when we consider the average amount in the first six feet, is quite uniform from east to west, but in the most humid areas it increases rapidly from the surface to the sixth foot, below which the high proportion continues to at least twice this depth, while in the semi-arid areas it decreases from the surface downward. In the latter, however, if we allow for the neutralizing action of the carbonates the amount dissolved by the citric acid is quite uniform throughout the six feet.

The eastern soils and subsoils contain only very small amounts of carbonates but show a neutral reaction (5, p. 422). The western surface soils carry but little carbonate but the corresponding deeper subsoils are rich in this, 3.5 to 5.0 per cent.

In mechanical composition, (5, p. 407) and hygroscopicity (4, p. 215) the subsoils do not differ markedly from the surface soils.

In the case of the most humid areas the different factors that might be expected to affect unfavorably the growth of legumes on the subsoils in comparison with the surface soils would be the lower proportion of citric-acid-soluble potash, the poorer tilth due to lack of organic matter and the

lower nitrogen content, while the higher proportion of citric-acid-soluble phosphoric acid in the eastern subsoils would appear their only advantage. However, the very high content of total potash at all depths, the lessened influence of the tilth upon an established legume and the doubtful importance of the soil nitrogen content to such plants, might serve to make the advantage of the high available phosphoric acid content more than offset the apparently disadvantageous factors mentioned.

With the semi-arid western areas the marked changes in composition observed with increasing depth may fail to be distinctly beneficial. The increase in carbonate content with increase of depth is great but even the surface soils have sufficient, and the same may be said of the citric-acid-soluble potash. The nitrogen content decreases and the citric-acid-soluble phosphoric acid does not increase with depth.

Behavior to be Anticipated from Composition

When we consider the composition of the soils and the climate of the region it is evident that we should expect but little downward movement of the soil colloids. The soils are comparatively rich in soluble salts, the carbonate content of the subsoils is in some cases high and in all at least sufficient for calciphilous plants. The precipitation occurs chiefly as summer rains (4, p. 206-214), a large part in the form of thunderstorms of brief duration, causing a large loss of water as run-off. Falling as it does during the growing season only a very small part of it penetrates far into the subsoil, it being almost wholly lost by run-off, direct evaporation or transpiration. The portion that does penetrate to any considerable distance will, on account of the high temperature, the carbonate and soluble-salt content of the soil, be well charged with carbonic acid and electrolytes, thus preventing the deflocculation of the soil colloids. The dissolved substances carried down in the rain will, in the virgin prairies, be largely returned, partly through the plant roots to be left lying upon the surface on the death of the aerial parts, and partly through capillarity. While the effect of the latter in elevating moisture from the deeper subsoil to the surface is slight compared with the total amount so moved, it probably is quite sufficient to maintain a fairly uniform salt content throughout the upper few feet of soil, when the climate is such as to cause almost no percolation. The salts would tend to move downward during the warm, rainy portion of the year and upward during the dry and cooler months.

Thus both climate and soil composition are such as to hinder the translocation of the soil colloids and the climate is such as to favor the maintenance of the present soil composition. The water resulting from the melting of the snow is not to be expected to affect seriously the soil colloids. The total snowfall is light and much of what falls is blown by the wind into depressions. At times, falling upon deeply frozen soil, it is lost as run-off when it melts. Thus unusually heavy snow-falls may produce

disastrous floods without contributing much moisture to the subsoil. Even where the snow falls upon the unfrozen soil and does not drift, the resulting water rarely penetrates beyond the reach of annual crop plants.

The mechanical composition of the soil and subsoil as shown both by actual mechanical analysis and by the hygroscopicity, indicates that no great downward translocation of colloidal clay and very fine silt has taken place, although in the eastern areas the hygroscopic coefficients reveal an appreciable increase in the fineness of texture in passing from the first to the twelfth inch. This is shown in Table I. The "clay," as determined by the centrifugal method, included all silt particles under 0.005 mm. diameter and these formed a large proportion of the separate.

Pot Experiments

In the pot experiments we used 31 different bulk samples of soil collected from different parts of Nebraska (fig. 1), all being from the loess-

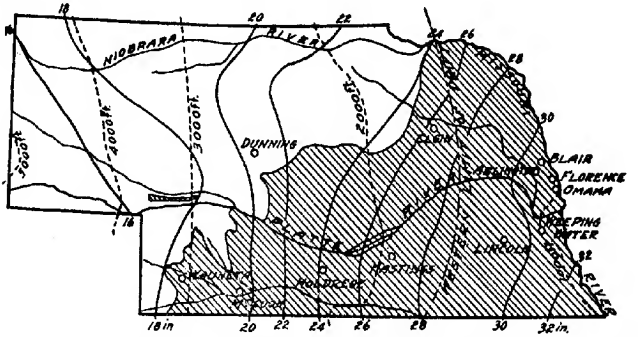

Fig. 1.—Map of Nebraska, showing sources of samples, distribution of the loess (shaded), annual precipation, elevation and westward limit of the glacial drift.

covered portion, except one, a dune sand. Twenty-nine of the samples corresponded to, but were not identical with, the samples of loess soils reported in the earlier papers of this series (3, 4, 5). The sets of soils from Lincoln, Hastings, Holdrege, McCook and Wauneta, respectively, were taken each from a single place instead of being composites from five different fields (4, p. 204). A pit was dug in the grass-covered border of the roadway, and about 150 pounds of soil taken from each of the first six foot-sections. The land where the pit was dug had received little or no manure, and had not been cultivated in recent years. When these bulk samples reached the experiment station they were stored in sacks in a dry room for three or four months, after which they were reduced to a fine state of division by a heavy wooden tamping-block and well mixed by being several times passed through a ⅛-inch screen (1, p. 80), which served at the same time to remove most of the roots. The samples had been collected for another experiment, previously reported, (1, p. 78-108) and the

greater portion of each had been used for this purpose before the present experiment was decided upon. The quantity remaining of some was too small to permit of filling the pots as full as desirable and in the case of the first-foot samples from Lincoln and Holdredge only a few pounds remained. The latter of these two was omitted throughout the experiment, while for the former we later introduced surface soil from a long-cultivated field on the experiment station farm, the "Surface Soil" used in previously reported experiments (1, 80, 83).

TABLE I

HYGROSCOPIC COEFFICIENTS AND CLAY CONTENT OF LOESS SOILS FROM DIFFERENT LEVELS

HYGROSCOPIC COEFFICIENTS

	Humid Areas[1]	Intermediate Areas[2]	Semi-Arid Areas[3]
First inch	11.5	10.9	8.5
Second inch	11.1	10.0	8.2
Third inch	11.0	9.4	8.3
Fourth inch	11.1	9.0	8.3
Fifth inch	11.3	8.8	8.4
Sixth inch	11.5	9.2	8.9
Seventh inch	11.7	9.6	9.1
Eighth inch	12.1	9.7	9.3
Ninth inch	12.6	9.7	9.2
Tenth inch	12.6	10.3	9.5
Eleventh inch	12.7	10.1	9.6
Twelfth inch	12.9	10.2	9.4
Second foot	14.0	11.4	10.2
Third foot	13.9	11.8	10.2
Fourth foot	13.0	10.6	9.8
Fifth foot	12.6	10.1	9.0
Sixth foot	12.5	10.0	8.7

CLAY

	Per cent	Per cent	Per cent
First foot	14.3	7.1	7.0
Second foot	19.2	11.8	8.0
Third foot	18.9	12.0	7.9
Fourth foot	18.2	10.2	8.4
Fifth foot	17.0	7.9	5.6
Sixth foot	16.8	7.8	5.4

[1] Weeping Water and Lincoln. [2] Hastings and Holdrege. [3] McCook and Wauneta.

The soil from near Elgin was collected especially for this experiment. A well was sunk to a depth of 20 feet in the loess which at this place had a thickness of 35 feet, and the sample at once collected from the bottom of this well. The field had been under cultivation about thirty years, during the last ten of which it had been in alfalfa. The water-table was probably about 25 to 30 feet below the depth from which the sample was taken. The alfalfa roots extended below the bottom of the well, and a moisture study (1, p. 118) indicated that they were still numerous enough at 30 feet below the surface to reduce the available soil moisture to a very low point.

The sample of dune sand was collected from the bare surface of a "blow-out" near Dunning.

The nitrogen content and the hygroscopic coefficients of the samples are reported in Table II, as is also the dry weight of each used in the experiment with alfalfa. Previous to the planting of this crop eight of the jars had contained from 2 to 5 pounds more, but finding these too full to water conveniently we lessened the amount at the time the soil was being prepared for alfalfa. In the case of seven of the soils we found at the conclusion of the experiment, when we went to make the nitrogen determinations, that we had no samples of the original soil. That remaining from the pot experiments had become so mixed with quartz sand and dune sand, especially due to the removal of the roots as described below, that it was not sufficiently similar to the original soil to justify the use of the data from it in the present instance. Accordingly we have introduced for these in the table the nitrogen percentages found in the composite samples from the corresponding areas (4, p. 221). The nitrogen percentages of the respective soils remaining in the pots were lower than these data, and, allowing for the admixture of sand, as indicated by changes in the hygroscopic coefficient, would indicate that the soils as introduced into the pots were not dissimilar in composition to the corresponding composites.

We employed 4-gallon ice-water jars, 12.6 inches high and 8.8 inches in diameter, with a hole at the side 1.0 inch above the bottom, all measurements being made on the inside. To facilitate the drainage a mixture of 2.5 pounds of coarse crushed limestone and 2.5 pounds of dune sand was placed in the bottom of each, thus permitting water which accumulated in the open substratum to escape freely.

While the pots were kept in the greenhouse they were watered with tap-water from a deep well on the experiment station farm. An analysis showed that this water contained practically no phosphoric acid and only a very small amount of potash, in an acre foot of the water amounting to 17.67 pounds, enough to supply only about half of that contained in one ton of alfalfa hay. The water carried a very considerable amount of calcium bicarbonate, but the soils, with the exception of the dune sand, were already so well supplied with lime that there appears no reason to attribute to this additional amount any beneficial effect upon the growth of the crops.

The jars, except those of the Elgin subsoil and dune sand, were filled on April 25, 1911, thoroughly moistened, inoculated by means of an extract from a clover soil and planted to red clover. A good stand of plants was secured, but during the following five weeks, in order to secure the inoculation of the plants on part of the subsoils it was necessary to give these several additional treatments. In such cases the same amount of soil extract was added to each pot, including those in which the plants already gave every evidence of inoculation. In each case we used for the

TABLE II

SOILS USED IN POT EXPERIMENTS

Depth Foot	Nitrogen Per cent	Hygroscopic Coefficient	Weight of Soil Used in Pot Exper'ts: pounds
LINCOLN			
First	.244	12.1	*
Second	.106	15.3	30
Third	.058	14.6	29
Fourth	[1].060	13.2	28
Fifth	[1].042	13.2	26
Sixth	.041	13.5	28
HASTINGS			
First	.172	10.7	30
Second	.102	13.2	27
Third	.056	11.7	32
Fourth	.032	11.5	32
Fifth	.035	10.8	30
Sixth	.025	10.5	27
HOLDREGE			
Second	[1].101	11.7	28
Third	[1].065	12.0	27
Fourth	[1].045	10.6	32
Fifth	[1].034	9.7	30
Sixth	[1].034	9.6	27
McCOOK			
First	.142	9.4	30
Second	.071	11.0	35
Third	.040	9.6	35
Fourth	.030	9.0	30
Fifth	.027	8.8	35
Sixth	.022	8.4	30
WAUNETA			
First	.148	9.2	30
Second	.080	9.5	30
Third	.052	9.9	35
Fourth	.037	10.1	30
Fifth	.032	9.5	35
Sixth	.029	8.6	32
ELGIN			
Twentieth	.028	9.9	
DUNE SAND			
First	....	0.6	

* Datum lost. [1] Data from composite samples from prairie fields.

purpose a somewhat turbid extract from a yellow subsoil that was exposed in a railway cut, and upon which there was a good growth of red clover. On May 29, when the pots were all removed to the open air, the appearance of the plants on each was recorded. The plants were large and of good color on all the Lincoln soils, on all those from Holdrege except the second foot, on the first foot from Hastings, McCook and Wauneta, and on the second foot from the last mentioned place. They were small, but of good color on the second foot from Holdrege and on the second and third foot from Hastings, while on the twelve other pots the plants were small and light yellow in color. The application of the soil extract on this date was effective, and a week later all the plants had a good color. The pots, exposed in the garden, received no water in addition to the rainfall. The hot, dry weather and the depredation of small animals soon reduced the plants in many of the pots to such an extent that the experiment was practically abandoned, but, on August 17, happening to visit the pots and observing the good growth on the Lincoln subsoil, photographs were taken of all on which plants survived (Pl. I). No further attention was given them until October 2, when we moved them to the greenhouse and removed all the clover plants, together with their roots to a depth of six inches.

As the record of the weights of the clover crop has been lost, the notations of May 29 and the photograph of August 17 constitute our only definite record of the relative growth on the different soils. The marked differences in appearance of the clover in the different pots shown in the photograph are due chiefly to the differences in the number of surviving plants, the individual plants having been quite similar in size. The Lincoln fifth foot showed a surprisingly good growth, the best of all.

The importance of the behavior of the clover crop in this experiment lies in its relation to two factors, viz: the influence of the tap water later employed and the weathering effect to which the subsoils were exposed between April 29, 1911 and March 9, 1912, the date upon which they were planted to alfalfa. Although between the time of planting the seed and that of taking the photograph the pots had received only three or four light waterings of tap water, and there had been little opportunity for weathering, the growth of the clover showed no dependence upon the depth from which the soil had been taken. Accordingly, it is probable that the alfalfa would have done as well in the jars of subsoil if it had been planted at once on this instead of over a year after the soil had been brought under experiment.

On October 12, ten days after the removal of the clover plants, the soil was thoroughly stirred to a depth of six inches and moistened. The jars containing the first, third and fifth foot samples were planted to corn and the others to wax beans. All were kept in the greenhouse and watered freely with tap water.

The soil in the pots planted to beans was inoculated and from the first the plants in all did well. In each pot two were allowed to grow. On November 22 the crop, not yet ripe, was removed, dried and weighed. The yields (Table III) indicate that the growth on the whole was as vigorous on the subsoil from one depth as from another. The growth in the Wauneta pots (Pl. II, fig. 1) was typical of all except that of the Lincoln fourth foot mentioned below.

As soon as the wax beans had been removed the jars were replanted to Mexican beans and teparies (8), five plants of each being left. The beans formed seed, but the teparies, while making a good growth of vine, failed to form any seed. On January 29, 1912, the pots were photographed (Pl. II, fig. 2) and on February 15, the beans being ripe, the crop was harvested, dried, weighed and the beans counted (Table III). A satisfactory growth was obtained on all the jars except the Lincoln fourth foot, on which no beans and only two tepary plants survived; this pot had also shown the poorest growth of wax beans.

The Elgin subsoil, secured on August 12, 1912 was like the other soils placed in jars with a substratum of crushed rock and dune sand, and on November 22, two of these pots were planted to beans and teparies, inoculation being provided for. The stand of teparies was unsatisfactory on these, the one pot having six beans and four teparies and the other only six bean plants when photographed on January 29 (Pl. II, fig. 3). The growth of the plants was satisfactory.

The corn made a rather slow growth and during late December and January remained almost stationary, the weather being so cold that we could not keep the greenhouse warm. On January 10, 1912, the corn was photographed (Pl. III, fig. 1). Soon after this the tops of the tallest plants were severely injured by frost and very little growth was to be observed between the time the photographs were taken and February 9, when they were cut off level with the surface of the soil, dried and weighed (Table III).

Corn planted on two jars of Elgin subsoil at the same time as the beans and teparies was allowed to grow until March 29, when it was photographed (Pl. III, fig. 2) and removed. The growth was as poor as on any of the subsoils.

The conduct of the beans and teparies shows that their growth was independent of the depth from which the subsoil had been taken, at least to 6 feet.

The growth of the corn was what was to be expected on subsoils from humid regions. From both this experiment and our field observations on corn and wheat growing on badly eroded parts of fields we fail to find any ground to consider the loess subsoils of the Transition Region to be any less unproductive of non-leguminous plants than has been found on humid soils in regions of heavier winter precipitation.

TABLE III

YIELD FROM THE DIFFERENT POTS PREVIOUS TO PLANTING ALFALFA

Depth Foot	Beans Nov. 22, 1911	Beans and Teparies Feb. 15, 1912		The Two Crops of Legumes	Corn Feb. 8, 1912
	gm.	gm.	No. of Seeds	gm.	gm.
LINCOLN					
First	...	...	..	....	72.0
Second	5.6	7.0	16	12.6	....
Third	...	...	..	....	14.3
Fourth	1.3	*	0	....	....
Fifth	...	...	..	....	28.2
Sixth	3.2	8.3	21	11.5	....
HASTINGS					
First	...	...	..	....	42.4
Second	2.3	7.6	19	9.9	....
Third	...	...	..	....	7.5
Fourth	2.6	8.2	21	10.8	....
Fifth	...	...	..	....	2.6
Sixth	4.2	6.9	16	11.1	....
HOLDREGE					
Second	2.6	8.4	23	11.0	....
Third	...	...	..	....	16.4
Fourth	3.6	7.4	16	11.0	....
Fifth	...	...	..	....	10.5
Sixth	3.7	*	12	....	....
McCOOK					
First	...	...	..	....	43.9
Second	4.6	7.4	15	12.0	....
Third	...	...	..	....	8.3
Fourth	4.3	8.2	19	12.5	....
Fifth	...	...	..	....	2.5
Sixth	3.3	8.3	18	11.6	....
WAUNETA					
First	...	...	..	....	110.6
Second	6.4	8.2	18	14.6	....
Third	...	...	..	....	27.2
Fourth	4.8	6.6	14	11.4	....
Fifth	...	...	..	....	13.6
Sixth	3.9	7.6	13	11.5	....
ELGIN SUBSOIL					
A	...	*	9	....	....
B	...	*	7	....	....

* Datum lost.

On March 9, 1912, we planted in all the jars previously employed, and in addition one filled with dune sand, two rows of alfalfa and two of medium red clover. A week later, it having become evident that the seed in the first foot samples was germinating much more rapidly than in the others, evidently due to the black soil's absorbing more heat, the windows of the greenhouse not being whitewashed, we added a quarter-inch layer of coarse quartz sand to the surface of each pot. After this the plants developed with equal rapidity in all. To inoculate the alfalfa plants we employed the extract from an exposed subsoil on which alfalfa plants were growing. By April 10 it was evident that all plants were inoculated, and the number in each pot was reduced to 16 of alfalfa and 16 of clover. All of the clover plants thus removed were saved, dried and included with the crop removed on May 1, while those of the alfalfa were included with that of June 6.

From the time of planting the alfalfa and clover until the experiment was brought to an end on June 10, 1913, the pots were kept in the greenhouse and watered freely with the tap water referred to above. Nine crops in all were secured, one of clover and eight of alfalfa (Table IV).

On May 10, all the clover plants, which averaged about nine inches in height, were removed by pulling out of the thoroughly wetted soil, so that the roots also were secured. The yields of dry matter of the clover crop as reported in Table IV include the roots as well as the tops of the clover. In the Lincoln and Hastings pots the yield of clover was comparatively good at all levels, but, as alfalfa plants were growing beside the clover these yields in themselves have little significance.

The alfalfa was harvested crop by crop as it came into bloom. Eight cuttings were secured during the 15 months that the experiment was continued, the dates being as follows: 1—June 6, 2—July 20, 3—August 19, 4—September 28, 5—November 30, 6—January 25, 7—April 7, 8—June 10. The yields are reported in Table IV. The pots bearing the first crop were photographed on June 6 (Pl. IV). The growth appears very similar on all except the fifth foot at McCook, the fifth and sixth at Wauneta, one of the pots of Elgin subsoil (D) and the dune sand. The subsequent crops showed a similar resemblance.

The fifth foot of soil from Wauneta very soon after being planted to alfalfa was observed to have become puddled to such an extent that it was almost impervious to water. The same was true of the Elgin subsoil D, and as with the latter the pot was purposely cracked to provide drainage, an improvement was soon observed; after the second crop of alfalfa, however, this pot was discarded.

After the second crop of alfalfa had been removed the Holdrege jars were devoted to another experiment without removing the roots of the alfalfa plants; hence the data for the Holdrege soils are fewer than for the others.

In the case of the eastern three areas there is little difference in productivity shown by the different levels. With one cutting the highest yield would be shown by one level, and with the next by a different level. Thus with the Lincoln pots the first foot gave the maximum yield with six crops, while better, or practically as good, yields were obtained with the fifth foot in three, with the fourth foot in two, with the sixth foot in two and with the second foot in one. With the Hastings pots the yields on the subsoils equal to or exceeding the yields on the surface foot occurred even more frequently. No connection between the yields and the weights of the soils employed (Table II) is evident. The whole of the data on these two areas would indicate that there is little difference in the productivity of the different levels, although the surface foot is probably slightly the most productive. The twentieth foot of subsoil from Elgin, while not producing as well as the eastern subsoils, which were from nearer the surface, did surprisingly well, the yield of the last three crops on B and C being but little below that of the corresponding crops on the surface soils. The dune sand, although watered daily so that the yield should not be limited by want of moisture, remained unproductive and was discarded after the fourth crop.

After removing the eighth crop of alfalfa all the pots were thoroughly soaked in water and the alfalfa roots removed. The soil was washed away with a jet of water, the roots dried at 105° C. and weighed. The total yields of the tops from the eight crops of alfalfa and one of clover (including the roots) are reported in Table V along with the weight of the roots. The ratio of tops to roots is distinctly higher in the surface foot than in the subsoils. When the yields are computed as tons per acre (Table VI) it is seen that they are not markedly dissimilar to those ordinarily obtained in the field from the same number of cuttings.

All the alfalfa from each pot was saved separately, it being placed in a fruit jar as soon as it had been dried and weighed. At the conclusion of the experiment, in order to determine the amount of nitrogen removed, we analyzed samples of the hay from the nine crops in ten pots, thus including also the roots of the clover plants, which, however, were so light that they would have no important influence upon the composition. Composites were made of the roots from the seven different levels and the nitrogen determined in these. The data are reported in Table VII. Assuming on the basis of these analyses a nitrogen content of 2.50 in the tops and of 2.00 in the roots we have calculated the weights of nitrogen in the leaves and stems of the nine crops, and in the roots remaining (Table VIII). In Table IX these data are calculated to pounds of nitrogen per acre, and in Table X to percentages of nitrogen in the soil.

The nitrogen content of neither tops nor roots seems distinctly dependent upon the level from which the soil was taken. Accordingly the weight of nitrogen in both showed as little dependence upon the depth of the level as did the dry matter. Computed to an acre basis it is seen that

TABLE IV

YIELD OF DRY MATTER FROM NINE CROPS, ONE OF CLOVER AND EIGHT OF ALFALFA

LINCOLN

Crop	First Foot gm.	Second Foot gm.	Third Foot gm.	Fourth Foot gm.	Fifth Foot gm.	Sixth Foot gm.
Clover	4.8	3.1	3.2	3.2	2.7	2.4
Alfalfa 1	9.5	7.1	7.4	9.2	6.1	6.9
Alfalfa 2	6.1	6.1	7.2	7.2	9.9	6.4
Alfalfa 3	13.7	10.0	9.9	11.8	12.5	12.1
Alfalfa 4	7.7	5.7	6.1	5.9	7.4	7.4
Alfalfa 5	7.3	4.6	6.3	7.3	7.9	8.0
Alfalfa 6	2.8	1.3	2.3	2.1	2.8	2.3
Alfalfa 7	11.4	5.4	5.0	7.3	8.3	6.5
Alfalfa 8	16.4	20.4	18.9	16.5	16.0	17.6
First 3	20.4	16.3	17.8	19.6	18.7	15.7
Second 3	28.7	20.3	22.3	25.0	27.8	27.5
Third 3	30.6	27.1	26.2	25.9	27.1	26.4
Total for 9...	79.7	63.7	66.3	70.5	73.6	69.6

HASTINGS

Clover	3.3	2.5	2.6	4.1	2.2	2.1
Alfalfa 1	6.2	5.9	8.5	6.9	9.2	7.8
Alfalfa 2	6.0	5.9	5.3	6.3	6.1	5.9
Alfalfa 3	12.0	11.8	12.7	13.1	11.0	8.5
Alfalfa 4	5.3	5.9	8.0	5.3	5.0	3.2
Alfalfa 5	6.8	6.1	8.0	6.1	6.6	3.8
Alfalfa 6	3.1	2.4	1.4	2.3	2.5	0.7
Alfalfa 7	3.4	6.2	5.3	6.1	5.6	5.3
Alfalfa 8	11.6*	17.9	21.2	18.3	19.8	20.5
First 3	15.5	14.3	16.4	17.3	17.5	15.8
Second 3	24.1	23.8	28.7	24.5	22.6	15.5
Third 3	18.1*	26.5	27.9	26.7	27.9	26.5
Total for 9...	*	64.6	73.0	68.5	68.0	57.8

HOLDREGE

Clover	....	2.1	6.1	3.7	2.2	1.8
Alfalfa 1	....	6.2	7.3	6.7	8.4	6.5

McCOOK

Clover	2.8	2.9	2.7	1.3	1.2	1.5
Alfalfa 1	7.5	5.8	6.5	4.6	2.9	8.2
Alfalfa 2	7.2	4.5	4.5	3.5	3.3	3.9
Alfalfa 3	11.8	10.2	8.2	3.7	3.6	6.0
Alfalfa 4	6.0	4.0	3.6	2.1	1.6	3.2
Alfalfa 5	2.6	4.5	3.6	1.2	1.2	2.3
Alfalfa 6	2.0	2.3	1.2	1.6	0.3	1.0
Alfalfa 7	5.4	4.6	4.8	2.9	2.3	2.6
Alfalfa 8	16.3	13.2	13.0	5.2	4.0	5.2
First 3	17.5	13.2	13.7	9.4	7.4	13.6
Second 3	20.4	18.7	15.4	7.0	6.4	11.5
Third 3	23.7	20.1	19.0	9.7	6.6	8.8
Total for 9...	61.6	52.0	48.1	26.1	20.4	33.9

TABLE IV—(Continued)
YIELD OF DRY MATTER FROM NINE CROPS, ONE OF CLOVER AND EIGHT OF ALFALFA

WAUNETA

Crop	First Foot gm.	Second Foot gm.	Third Foot gm.	Fourth Foot gm.	Fifth Foot gm.	Sixth Foot gm.
Clover	5.5	3.6	1.8	1.4	0.3	1.0
Alfalfa 1	5.7	6.8	5.3	2.7	1.2	2.6
Alfalfa 2	6.1	5.0	3.8	3.2	1.2	3.0
Alfalfa 3	9.8	10.3	8.5	4.6	1.3	3.5
Alfalfa 4	6.1	5.2	3.6	1.5	0.9	1.2
Alfalfa 5	7.6	5.1	3.9	2.1	0.5	1.4
Alfalfa 6	2.5	2.8	2.2	0.8	0.2	0.6
Alfalfa 7	4.5	5.5	3.5	2.1	1.1	1.2
Alfalfa 8	20.0	17.1	13.9	6.1	2.5	3.6
First 3	17.3	15.4	10.9	7.3	2.7	6.6
Second 3	23.5	20.6	16.0	8.2	2.7	6.1
Third 3	27.0	25.4	19.6	9.0	3.8	5.4
Total for 9...	67.8	61.4	46.5	24.5	9.2	18.1

	DUNE SAND	ELGIN SUBSOIL			
	gm.	A gm.	B gm.	C gm.	D gm.
Clover	0.8	1.4	2.4	1.6	2.1
Alfalfa 1 ..	1.1	5.5	6.5	4.3	4.8
Alfalfa 2 ..	2.5	3.7	4.2	3.5	1.5
Alfalfa 3 ..	4.2	6.6	8.3	5.9	...
Alfalfa 4 ..	...	3.0	4.1	3.8	...
Alfalfa 5 ..	...	2.9	4.4	2.8	...
Alfalfa 6 ..	...	1.8	2.5	2.6	...
Alfalfa 7 ..	...	2.3	4.8	3.6	...
Alfalfa 8 ..	...	10.0	15.6	15.7	...
First 3	4.4	10.6	13.1	9.4	8.4
Second 3 ...	...	12.5	16.8	12.5	...
Third 3	...	14.1	22.9	21.9	...
Total for 9.	...	37.2	52.8	43.8	...

* Incomplete, part of last cutting being lost.

from 100 to 150 pounds of nitrogen per acre had remained in the roots, much the same as found in the roots in the surface foot of old alfalfa fields (Table XI). However, the amount contained in the roots was so small in comparison with that originally in the subsoils that the increase in nitrogen would not have been detected by an analysis if the roots had been ground with the soil in which they had grown.

Field Observations

To anyone who travels much about eastern Nebraska on the railways there will be presented many opportunities of seeing on the exposed subsoil in the railway cuts alfalfa, sweet clover and red clover growing luxuriantly. However, in most of the cases where the exposed subsoil is supporting a good growth of these legumes there is one circumstance that seriously affects their value as evidence of the ability of the subsoil to

support a satisfactory growth of these plants, viz: that the plants are growing in such places that the rains and winds bring down a considerable amount of the surface soil, while in many places the stand of legumes is not sufficiently dense to test fully the ability of the subsoil to supply the potash and phosphoric acid requirements.

TABLE V

YIELDS OF DRY MATTER IN THE TOPS FROM THE NINE CROPS AND IN THE ROOTS AT THE END OF THE EXPERIMENT

Depth Foot	Lincoln gm.	Hastings gm.	McCook gm.	Wauneta gm.	Average gm.	Elgin Subsoil gm.
			STEMS AND LEAVES			
1	79.7	*	61.6	67.8	66.7	A 37.2
2	63.7	64.6	52.0	61.4	60.4	B 52.8
3	66.3	73.0	48.1	46.5	58.5	C 45.8
4	70.5	68.5	26.1	24.5	47.4	
5	73.6	68.0	20.4	9.2	42.8	
6	69.6	57.8	34.9	18.1	45.1	
			ROOTS			
1	18.9	*	18.1	18.6	....	A 16.6
2	20.5	29.0	24.7	22.3	24.1	B 33.1
3	21.3	23.3	24.5	28.7	24.4	C 23.8
4	34.4	30.9	12.6	9.4	21.8	
5	30.5	28.6	14.1	3.4	19.1	
6	28.3	21.7	15.0	5.4	17.6	
			RATIO OF STEMS AND LEAVES TO ROOTS			
1	4.2	...	3.4	3.6	....	A 2.2
2	3.1	2.2	2.1	2.7	....	B 1.6
3	3.1	3.1	1.9	1.6	....	C 1.9
4	2.0	2.2	2.1	2.6	....	
5	2.4	2.4	1.4	2.7	....	
6	2.5	2.6	2.3	3.3	....	

* Incomplete, part of last cutting and part of roots being lost.

There are numerous small cuts, especially along the Burlington Railway, in which, during the last few years alfalfa has been sown on the

TABLE VI

YIELD OF DRY MATTER IN THE HAY FROM THE NINE CROPS, COMPUTED AS TONS PER ACRE

Depth Foot	Lincoln	Hastings	McCook	Wauneta	Average	Elgin Subsoil
1	9.10	*	7.03	7.74	7.61	A 4.24
2	7.26	7.38	5.93	7.00	6.89	B 6.03
3	7.57	8.32	5.47	5.31	6.67	C 5.25
4	8.06	7.80	2.98	2.79	5.41	
5	8.42	7.77	2.30	1.04	4.88	
6	7.96	6.61	3.98	2.07	5.15	

* Incomplete.

exposed yellow loess subsoil and is doing well, but we have been able to learn of no other places in which there are exposures of such interest as those at Arlington, Florence and Blair, especially the last.

TABLE VII

NITROGEN CONTENT OF TOPS AND ROOTS OF ALFALFA

TOPS

	Nitrogen Per cent
Lincoln—First foot	2.49
Lincoln—Second foot	2.51
Lincoln—Fifth foot	2.44
Hastings—First foot	2.56
Hastings—Fifth foot	2.43
McCook—First foot	2.59
McCook—Fifth foot	2.56
Wauneta—First foot	2.63
Wauneta—Fifth foot	2.55
Elgin—Subsoil	2.40

ROOTS

Composite* from first foot	1.96
Composite* from second foot	2.10
Composite* from third foot	1.92
Composite* from fourth foot	1.93
Composite* from fifth foot	2.17
Composite* from sixth foot	1.99
Composite from Elgin subsoil	1.88

* From Lincoln, Hastings, McCook and Wauneta.

TABLE VIII

WEIGHT OF NITROGEN IN LEAVES AND STEMS OF THE NINE CROPS AND IN THE ROOTS AT THE END OF THE EXPERIMENT

STEMS AND LEAVES

Depth Foot	Lincoln gm.	Hastings gm.	McCook gm.	Wauneta gm.	Elgin Subsoil gm.
1	1.99	1.44	1.54	1.69	A 0.93
2	1.59	1.61	1.30	1.53	B 1.30
3	1.66	1.83	1.20	1.16	C 1.14
4	1.76	1.71	0.65	0.61	
5	1.84	1.70	0.51	0.23	
6	1.74	1.44	0.87	0.45	

ROOTS

1	0.37	*	0.36	0.37	A 0.33
2	0.41	0.58	0.49	0.45	B 0.66
3	0.43	0.47	0.49	0.57	C 0.47
4	0.69	0.62	0.25	0.19	
5	0.61	0.57	0.28	0.06	
6	0.57	0.43	0.30	0.11	

* Incomplete.

TABLE IX

WEIGHT OF NITROGEN IN LEAVES AND STEMS OF THE NINE CROPS AND IN THE ROOTS, COMPUTED AS POUNDS PER ACRE

STEMS AND LEAVES

Depth Foot	Lincoln Pounds	Hastings Pounds	McCook Pounds	Wauneta Pounds	Elgin Subsoil Pounds
1	452.3	.....	350.0	384.1	A 211.3
2	361.4	366.0	295.5	347.7	B 295.5
3	377.3	416.0	272.7	263.6	C 259.1
4	400.0	388.7	147.7	138.6	
5	418.2	386.4	115.9	52.2	
6	395.5	327.3	197.7	102.2	

ROOTS

1	84.0	.....	81.8	84.1	A 75.0
2	93.1	131.8	111.4	102.2	B 150.0
3	97.6	106.9	111.4	129.5	C 106.9
4	156.7	140.9	56.8	43.2	
5	138.6	129.5	63.4	13.6	
6	129.5	97.6	68.2	25.0	

TABLE X

NITROGEN IN THE TOPS AND ROOTS OF THE ALFALFA PLANTS EXPRESSED AS PERCENTAGE OF THE WEIGHT OF THE SOIL IN THE POTS

STEMS AND LEAVES

Depth Foot	Lincoln Per cent	Hastings Per cent	McCook Per cent	Wauneta Per cent
1	.....	.....	.0113	.0124
2	.0117	.0131	.0082	.0112
3	.0131	.0126	.0075	.0073
4	.0138	.0123	.0048	.0045
5	.0156	.0125	.0032	.0015
6	.0137	.0118	.0064	.0031

ROOTS

1	.....	.....	.0026	.0027
2	.0030	.0047	.0031	.0033
3	.0034	.0032	.0031	.0036
4	.0054	.0043	.0018	.0014
5	.0052	.0042	.0018	.0004
6	.0045	.0035	.0022	.0007

Field at Railway Cut Near Arlington, Nebraska

About two miles east of Arlington a deep cut 1700 feet long and from 20 to 35 feet deep was made by the C. & N. W. Railway in 1868 (fig. 2). Part of the excavated loess was piled up on the high ground on the south side of the cut, about an acre and a half being covered to a depth of 15 to 18 feet by this mound of subsoil, which became covered with a growth of willows and weeds and so remained undisturbed until 1907, when the surface was worked down and seeded to alfalfa by the Marshall Brothers, who state that the alfalfa on this mound has since yielded as well as other

alfalfa fields in the vicinity except during unusually dry seasons. In the field itself there is a fair basis of comparison, as the alfalfa extends beyond the limits of the mound of subsoil, part being on typical black soil of the prairie-covered loess. The views shown in Plate V were secured at the end of August, 1912. Composite samples of the first six inches and

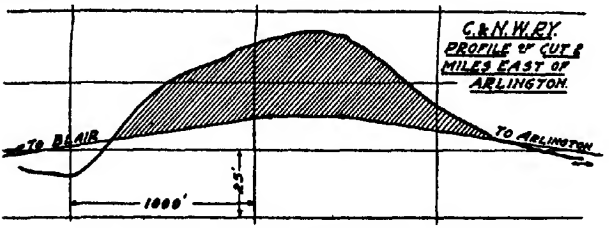

Fig. 2.—Profile of railway cut near Arlington, Nebraska.

of the second six inches of soil taken from the mound showed a nitrogen content of 0.061 and 0.038 per cent, respectively, compared with 0.228 to 0.245 per cent found in the surface 12 inches of virgin prairie fields on similar soil (4, p. 220).

Field in Excavation at Florence

Opposite the railway station at Florence is an excavation of about seven acres that was made near the settling basins used in clarifying the muddy waters of the Missouri River for the adjoining city of Omaha (Pl. VI). On the side next to the river more than 20 feet of soil was removed in 1890. In the following year the rough surface was plowed deeply, leveled, thoroughly harrowed, and the whole field of 17 acres including the excavated area seeded to oats. In the spring of 1892 the field was seeded to alfalfa and since has been continuously in this crop. At first on the lower part of the excavation, water and ice stood for much of the winter and killed the crop. A drainage ditch was then dug and this portion reseeded, since which time no reseeding has been necessary. We are assured by the water-works officials that the field has from the very beginning given satisfactory yields of hay, four crops being cut each year.

Fields in Excavation at Blair

Adjacent to the town of Blair an excavation, covering over 13 acres and averaging 25 feet in depth, being from 30 to 35 feet over a considerable portion, was made to secure material for the approaches to the railway bridge over the Missouri River. As the Blair depot is situated to the west on the natural surface, and a low plain lies to the east, the work of excavation consisted essentially of planing off level with the railway grade about eight acres on the south side of the track and five on the north (fig. 3 and 4). The loess at this point extends probably 25 feet below the bottom of the excavation.

For the history of the fields previous to the autumn of 1811 we are indebted to C. & N. W. Railway officials, to Mr. John Aye and to Mr. C. H. Grimm, the latter of whom for nine years has owned the eight acres on the south side of the cut, and who for the past forty years or more has lived on the farm, immediately adjacent to this.

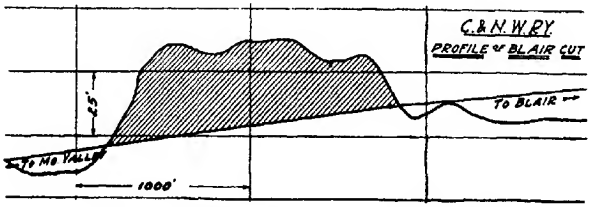

Fig. 3.—Profile of railway cut at Blair, Nebraska.

The original cut, just wide enough to permit of a single track was made in 1868 (fig. 4). Between 1883 and 1887 the excavation was made on the south side of the track. The subsoil was left very rough, and for some years no crop was grown upon it, but, during the next five years patches of sorghum, beans and corn were planted. All of these, except the beans gave very poor yields. In 1896 Mr. Grimm leased the field, leveled and seeded it to alfalfa, much of which killed out during the following winter, probably due to a lack of inoculation. The next season, it was reseeded and during the following 13 years each season gave good yields of alfalfa hay.

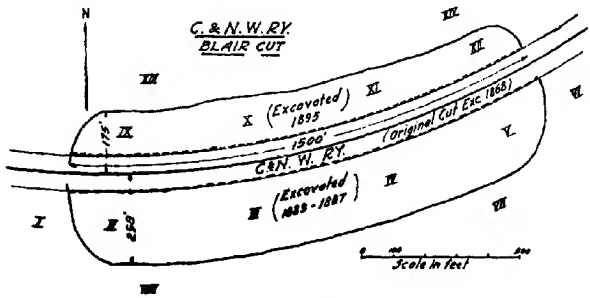

Fig. 4.—Diagram of railway cut at Blair, Nebraska, showing area of the two fields in the cut, the date and extent of the excavations made at different times and the source of the different sets of soil samples (I to XIV) analyzed.

Mr. Grimm, who in the meantime had become owner of the field, in 1909 gave part of it a heavy dressing of manure from his feed-yard, which is situated a short distance west of the west end of the cut, and planted it to corn in 1910. The object of applying the manure to the field was less to fertilize the field than to dispose of the manure. We have been unable to secure any satisfactory information as to the amount

of the manure applied to the whole of the field—eight acres—but much more was applied to the part nearest the feed-yard than to the other end. The yield of corn for the three years following was entirely satisfactory, in 1911, when an ear to the row trial was carried out on it, being about 50 bushels per acre; and in 1912 the yield was as good as on the best fields in the same county.

The large corn field belonging to the owner of the land on the south side of the cut extended onto the black soil at both the east and the west ends of the cut and covered the hill behind. The junction of the corn in the cut, that on the low ground at the west end and that on the sloping hill in the rear, is well shown in the accompanying photograph (Pl. VII, fig. 2). The crop on the low ground at both ends, with its deep black soil was no better than that in the cut, while that on the higher ground behind was not as good. On all three the same seed had been used and the care appeared to have been the same. Many other fields of corn in the same county were visited on the same day (August 21) but none better was seen.

From the standpoint of the investigation it is regrettable that any manure had been applied to the field previous to planting the corn in 1910. With no application the alfalfa had yielded well for 13 years, probably producing at least 40 tons per acre, as yields of less than three tons per acre are considered unsatisfactory in that locality. If the good growth of corn had been largely due to the application of manure we should have expected a more vigorous growth on the west half of the field. At the time, August 21, 1912, a careful comparison of the corn on the two ends was made to secure evidence of this but without finding that the crop was distinctly better on the one than on the other. This would make it appear that the fertility, as evidenced by the growth of the corn, was largely independent of the manure application, and due to the nitrogen supplied by the roots and residues of the alfalfa. From other studies (2, p. 59-62) it would appear that we might assume that the organic matter and nitrogen contained in the alfalfa stubble and the plant debris lying on the surface and in the alfalfa roots of the first two feet of soil would amount to about six tons of the former and 260 pounds of the latter per acre (Table XI).

The 5-acre portion of the cut on the north side of the railway track was excavated at a later date than that to the south, about 1895. Previous to 1913 it had never been manured or seeded at any time to alfalfa. For several years it had been planted to sorghum and corn without producing a satisfactory crop. The condition on August 21, 1912, is shown in the photograph (Pl. VIII, fig. 2). The crop was not worth gathering. Nowhere in Nebraska had we, that year, seen a poorer crop of corn. The views in Plate VIII show typical portions of the corn crop on the two fields. The south wall of the cut appears in the background of the first view and the north wall in that of the other.

In the spring of 1913 the west half of the field on the north side of the cut was seeded to alfalfa without a nurse crop, the alfalfa extending onto the undisturbed surface of what had been lower ground, just to the west of the cut. The east half of the field had been seeded to oats with alfalfa, this extending onto the ordinary surface just east of the east end of the cut. Thus on both halves of the field there was opportunity for a comparison of the growth of the crop on the exposed yellow subsoil with that on the black ordinary surface soil, the whole of each half being seeded by the same man at the same time and with the same seed. In figures 1 and 2 of Plate IX the dividing line between the two halves is shown distinctly.

TABLE XI

ORGANIC MATTER AND NITROGEN CONTAINED IN ROOTS OF ALFALFA PLANTS AND IN PLANT DEBRIS IN FIELDS WHICH HAVE BEEN IN ALFALFA 5 TO 8 YEARS

Depth Foot	Organic Matter per acre foot Pounds	Nitrogen per acre foot Pounds	Nitrogen in Soil Layer in the Form of Alfalfa Roots Per cent
ROOTS IN FIELD IN ALFALFA 8 YEARS			
1	5,069	104.9	0.00300
2	1,536	28.3	0.00080
3	806	14.7	0.00040
STUBBLE AND PLANT DEBRIS IN FIELD IN ALFALFA 5 YEARS			
...	5,478	126.0	.....

The alfalfa on both parts of the field was fully inoculated, a search failing to reveal a single plant without nodules. On the exposed yellow subsoil on the west half of the field, that without a nurse crop, the alfalfa, which had already been clipped, appeared everywhere almost as good as, but not better than, that on the black surface soil at the west end.

The oats on the east half of this field were light green in color, short and with the heads poorly filled on the yellow subsoil, but dark green, tall and with well filled heads on the black surface soil at the east end. The yield of dry matter on the latter would amount to five or ten times as much as on the former. There was a good stand of alfalfa on all parts of this, and the plants on the yellow subsoil were almost as large, although not as numerous as those on the west half, which bore no nurse crop, while on the black soil at the east end the plants were few in number, and small, evidently having succumbed to the oats in competition for moisture. Thus, where oats and alfalfa had been sown together the former was good on the black soil and poor on the exposed subsoil, while the latter was good on the subsoil and poor on the black soil. On the other hand, where the alfalfa had been sown without a nurse crop the alfalfa was as good on the black soil as on the exposed subsoil.

The railway tracks throughout the cut were bordered on both sides with a rank growth of volunteer sweet clover, which, on July 8, was as high as a man's head. This may be seen in the middle distance of figures 1 and 2, Plate IX.

For the crop returns from the two fields since July, 1913, we are indebted to Mr. Grimm. In 1913, a year of drouth in that locality, the potato crop on the south field, like that on the neighboring fields, was light. In the following three years it was planted to corn and yielded as well as other fields. On the north field a good stand of alfalfa was secured in 1913 and this has since given satisfactory crops of hay each season.

Thus the growth and yields of alfalfa on the north side of the cut have been satisfactory from the first planting without previous fertilization of any kind; according to what is to be regarded as strictly reliable evidence the same is true of the south side. The growth of non-leguminous crops on both sides has been extremely poor when they preceded the growth of alfalfa. For at least three years after plowing up the alfalfa stubble the corn was as good as on the ordinary fields with black soil, and at least in 1912 even much better than that on the adjacent fields with such soil. This good growth of corn appears to have been due essentially, so far as the nitrogen supply is concerned, to the effect of the alfalfa roots, stubble and plant residues.

Nitrogen Content of the Exposed Subsoil in the Blair Fields

In November, 1912, 14 sets of soil samples were taken at the cut, four from the portion north of the track, four from that south of it and the others from the surrounding land, which had not been disturbed in the course of the excavations. Of the latter, two were from the low ground at the ends of the south field, two from the high ground on the south and two from the high ground on the north. The source of each set is indicated in figure 4. Each sample of the first six inches and of the second six inches was a composite from 20 borings, while the samples of the second and third feet were composites from 5 borings. The data are reported in Table XII.

The nitrogen was much lower in the soil of the fields in the cut than at corresponding depths on the adjacent high land. In the surface six inches of the south field it was distinctly higher than in the north field, but still only about half as high as on the high land shown in the background of Plate VII, figures 1 and 2. It appears reasonable to assume that the original content of nitrogen in the subsoil when it was first exposed by excavation was between 0.020 and 0.050 per cent (4, p. 220). If one were to have expressed an opinion as to the productivity of the field, basing this upon the analyses, he would certainly have decided that a silt loam with only 0.10 per cent of total nitrogen would be unproductive, considering that similar soils in a virgin condition contain from 0.237 to

0.318 per cent (4, p. 222-223). The hygroscopic coefficient, 10.0, would indicate that the mechanical composition is similar to that found in the loess soils near Holdrege (5, p. 407).

The data in Set X, would indicate that in leveling the field some of the subsoil from the cut had been scraped out over the black soil of the low ground at the east end and had later become mixed with this.

TABLE XII

TOTAL NITROGEN IN SAMPLES FROM THE BLAIR RAILWAY CUT AND FROM THE ADJACENT FIELDS

Location		Nitrogen			
		Surface 6 inches Per cent	Second 6 inches Per cent	Second foot Per cent	Third foot Per cent
In the Cut					
South side	Set I	.125	.057	.037	.024
South side	Set II	.107	.051	.024	.022
South side	Set III	.103	.058	.035	.024
South side	Set IV	.101	.058	.030	.019
	Average	.109	.056	.031	.022
North side	Set XI	.079	.065	.065	.029
North side	Set XII	.069	.054	.031	.025
North side	Set XIII	.072	.054	.036	.025
North side	Set XIV	.066	.070	.057	.035
	Average	.071	.061	.047	.028
High Ground Outside the Cut					
South of west end	Set V	.207	.166	.121	.067
South of east end	Set VI	.215	.171	.105	.064
North of west end	Set VII	.186	.180	.128	.077
North of east end	Set VIII	.191	.169	.133	.080
	Average	.200	.171	.122	.072
Low Ground Outside the Cut					
At west end	Set IX	.203	.200	.146	.100
At east end	Set X	.158	.146	.250	.160
	Average	.180	.173	.198	.130

A determination of the citric-acid-soluble phosphoric acid of a composite of the surface six inch samples from the south side of the cut showed 0.057 per cent, an amount characteristic of the lower levels of the loess subsoils of eastern Nebraska (3, p. 312).

The nitrogen in the lower sections from the south side of the cut is lower than that in those from the north side. It might have been expected that the reverse would have been found on account of the growth of the alfalfa for 13 years. There appears no evidence to support the view that this crop appreciably increases the nitrogen content of the subsoil. The roots of the alfalfa plants once established continue year after year, adding but little nitrogen and organic matter until the plants either die out or

are killed by plowing. An earlier study (2, p. 60) showed that in a field that had been in alfalfa eight years the organic matter and nitrogen contained in an acre-foot of soil, estimated to weigh 3,500,000 pounds, was as shown in Table XI. Thus, even in the first foot the amount contained in the roots would be too small to be detected by determining the total nitrogen.

TABLE XIII

NITROGEN CONTENT OF A FIELD WHICH HAD BEEN IN ALFALFA 13 YEARS AND IN CEREAL CROPS 5 YEARS, COMPARED WITH THAT OF VIRGIN PRAIRIES.

Depth Foot	Old Alfalfa Field Per cent	Five Prairie Fields		
		Highest found in any of the five Per cent	Lowest found in any of the five Per cent	Average of the five Per cent
1	.207	.245	.234	.240
2	.125	.145	.122	.129
3	.073	.073	.063	.069
4	.051	.065	.050	.060
5	.038	.047	.036	.042
6	.034	.054	.036	.043
Average	.088	.103	.094	.097

One of the fields on the Nebraska Experiment Station Farm, which offered a good opportunity for the study of the effect of alfalfa upon the subsoil, furnishes data (Table XIII) confirmatory of the above view. That field had been under cultivation a little over 40 years, alfalfa having been grown on it from 1895 to 1907, giving an average yield of about 4 tons per acre. During the following five years it had been devoted to corn and small grains, the alfalfa roots thus being given an opportunity to decay and become an integral part of the soil mass. In 1912 composite samples were taken from each of the first six foot-sections, 15 borings well distributed over the field being made. The total nitrogen contained in the different sections compared with those from virgin prairie fields of the same locality (4, p. 220), shows that the surface foot is lower in nitrogen than in any of the prairie fields, while in the lower foot sections, that in the alfalfa field lies practically within the limits found for the virgin prairies.

Do Semi-Arid Subsoils Occupy an Intermediate Position?

It might be expected that the subsoils of the semi-arid region would show toward non-leguminous plants an intermediate behavior, being less unproductive compared with the surface soil than are the subsoils of humid regions. This is evidently the view implied by Lyon, Fippin and Buckman (13, p. 82). In the course of our studies on the Nebraska loess we have found no experimental evidence to support this. Our attention had been early called to this question by Hilgard, who, in 1907, in a letter to one of the authors, described the growth of non-leguminous plants—oats, barley, ray-grass, spurrey and dock—on a pile of freshly excavated subsoil in Berkeley and inquired whether there were any observations as to the existence of similar conditions in the western part of Nebraska.

Summary

Pot experiments, carried out with loess surface soils from the Transition Region and with the corresponding subsoils from the second to the sixth foot, using corn, beans, clover and alfalfa, showed that, while the subsoils were unproductive with corn, they evidenced no "rawness" toward the inoculated legumes. Eight successive crops of alfalfa gave almost as heavy yields on the subsoils from the eastern half of Nebraska as on the corresponding surface soils.

From field observations on the loess soils of eastern Nebraska it is evident that the deep subsoils at depths of even 20 to 30 feet, both when the surface of these is simply exposed by grading operations and when such excavated material is piled up, are not "raw" toward inoculated legumes, although very unproductive with non-leguminous crops. In the case of alfalfa they produce practically as satisfactory crops as the adjacent surface soils. After such exposed subsoils have for a period of years been devoted to the production of alfalfa hay it appears that they may be planted to non-leguminous crops with satisfactory returns.

There is a distinct increase in the nitrogen content of the surface portion of such exposed subsoils, it being much greater when the land is devoted to alfalfa. Even in this case, however, it has by no means attained that of the surrounding surface soils by the time its productivity for non-leguminous crops appears to have been restored. At depths below the plowed area, however, there appears to be no greater gain in nitrogen in the land devoted to alfalfa.

A field with ordinary surface soil devoted to the production of alfalfa hay for 13 years showed no distinct enrichment in nitrogen in the subsoil of the second to sixth foot.

The climate of the Nebraska portion of the Transition Region and the composition of the loess soils in it do not favor a concentration of soil colloids in the subsoil, such as that to which the "rawness" of the subsoils of humid regions in general is attributed.

The loess subsoils of the semi-arid part as well as those of the more humid half, of the Nebraska portion of the Transition Region show, in the matter of "rawness," a behavior intermediate between that of arid subsoils and that of the humid subsoils of the eastern and southern United States and of Western Europe, with inoculated legumes resembling the former, but with non-leguminous plants, the latter.

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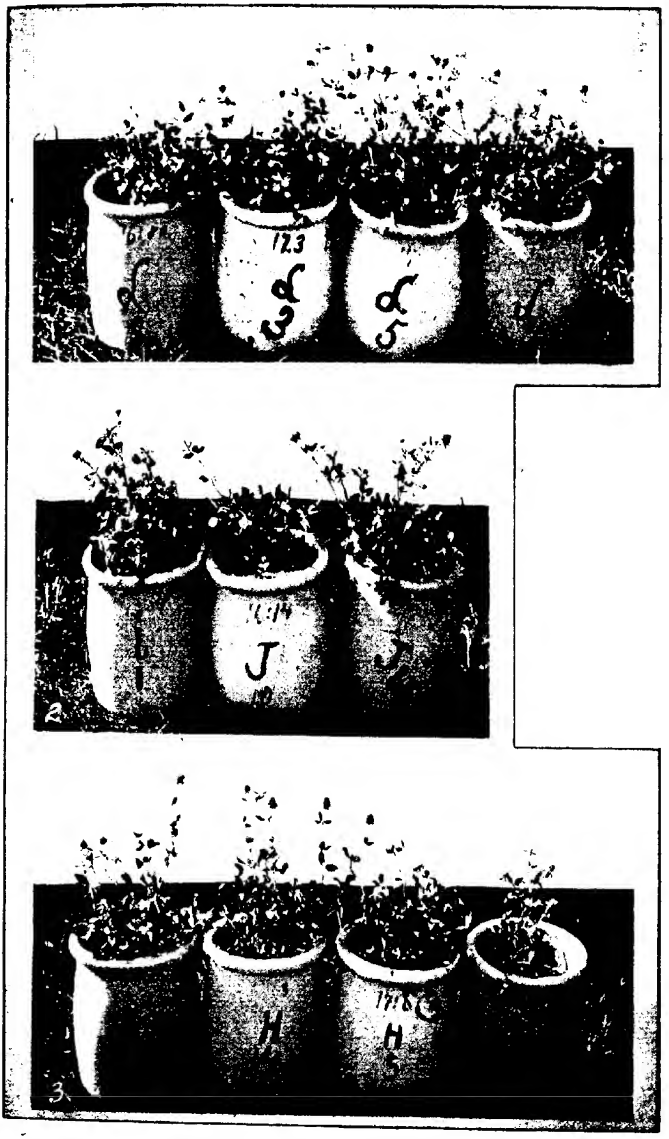
PLATE I

Red Clover, four months after planting, on soil taken from different foot levels at Lincoln (L), Hastings (J) and Holdrege (H).


12.3
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J
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PLATE II

Fig. 1.—Wax beans, six weeks after planting, on subsoils from the second, fourth and sixth foot at Wauneta.

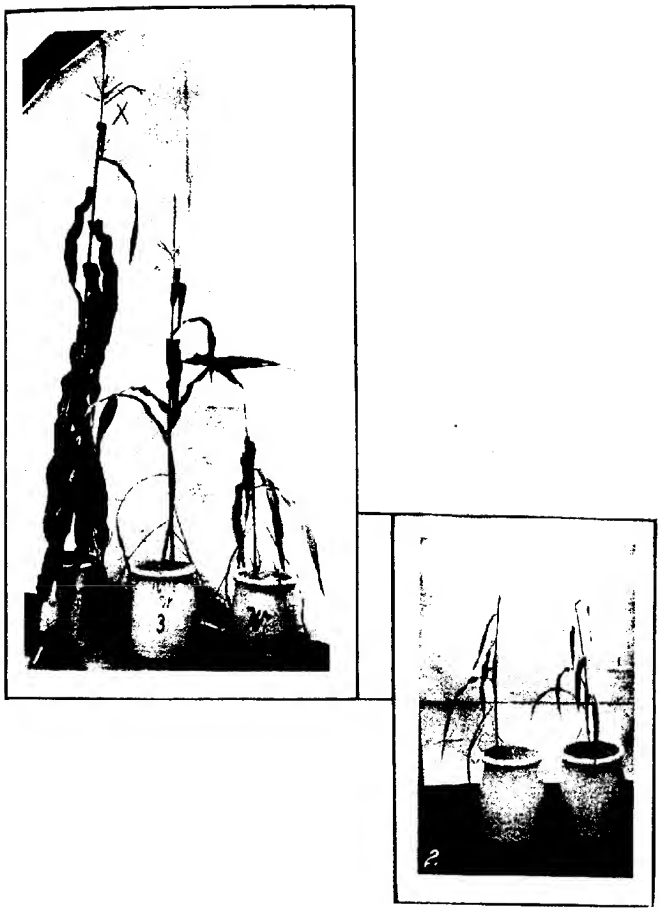
Fig. 2.—Mexican beans and teparies, ten weeks after planting, on the same soils as the preceding.

Fig. 3.—Mexican beans and teparies, ten weeks after planting, on the twentieth foot of subsoil from Elgin.

PLATE III

Fig. 1.—Corn, thirteen weeks after planting, on soils from the first, the third and the fifth foot at Wauneta.

Fig. 2.—Corn, fifteen weeks after planting, on the twentieth foot of subsoil from Elgin.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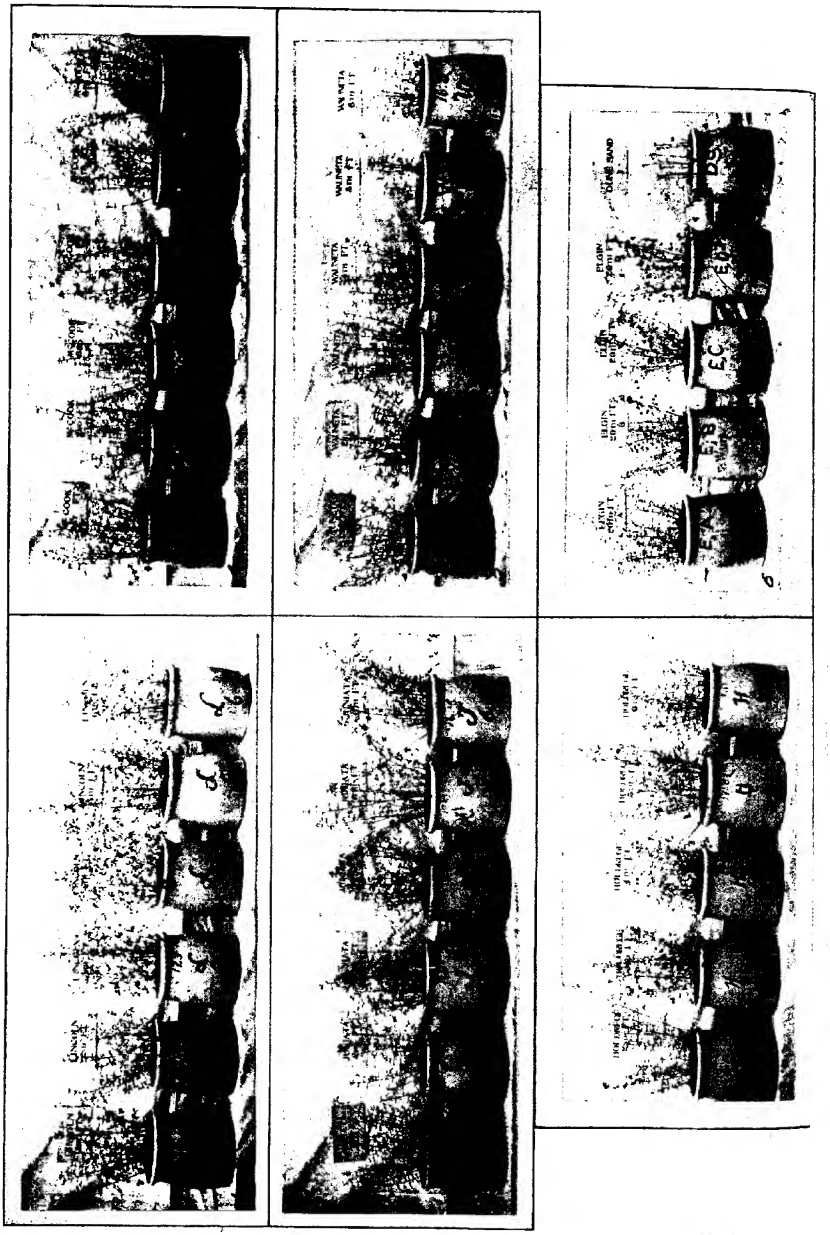

PLATE IV

First crop of alfalfa on soil taken from different levels at Lincoln (L), Hastings (or Juniata, J), Holdrege (H), McCook (M), Wauneta (W) and from the twentieth foot at Elgin (E) and on dune sand (D. S.).

PLATE V

Alfalfa on mound of subsoil at Arlington, Nebraska

Fig. 1.—Railway cut from which the excavated loess subsoil was removed.

Fig. 2.—Field of alfalfa on mound of subsoil 8 to 15 feet in depth, removed from the cut shown in figure 1. In the left background may be seen the trees which appear on the right in figure 1.

Fig. 3.—Same field as the above, showing alfalfa on mound of subsoil at the left and on ordinary black surface soil at the right between the fence and the hedge. As both the fence posts and the hedge are on the original surface the gradual increase in the depth of the subsoil to the left is evident.

Fig. 1

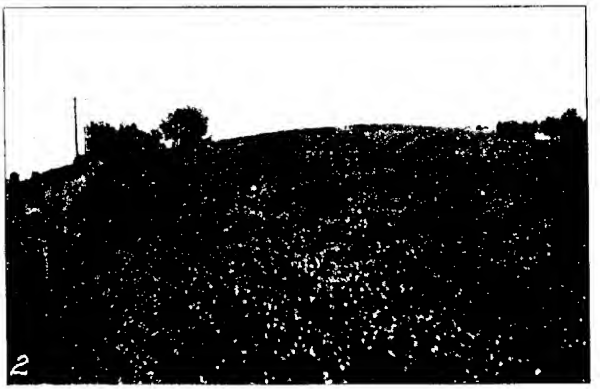

Fig. 2

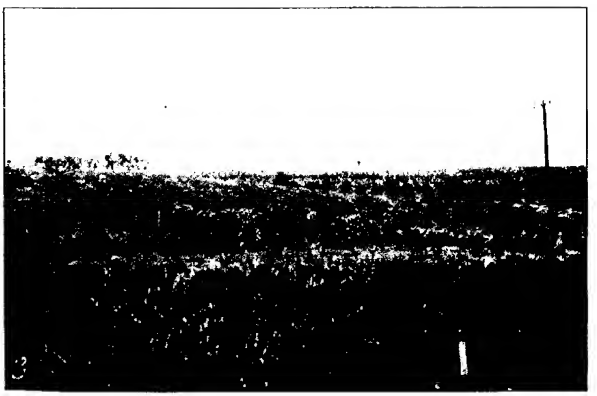

Fig. 3

Fig. 1

Fig. 2

PLATE VI

Alfalfa field in the excavation at Florence, Nebraska

Fig. 1.—The original level is shown in the background. Large cottonwood trees have grown at the foot of the artificial cliff.

Fig. 2.—A nearer view of the cliff and one of the cottonwood trees, showing the depth to which the soil was removed before the seeding to alfalfa.

PLATE VII

Fields in railway cut at Blair, Nebraska

Fig. 1.—East portion of south field, showing good corn crop in foreground, 30-foot wall of exposed subsoil in middle distance and corn field on ordinary surface soil in the background. August 21, 1912.

Fig. 2.—North field in foreground with west end of south field shown beyond the railway track. Both are in corn which also covers the hill in the background and extends down over the slope to join both that on the exposed subsoil and that on the low ground at the west end of the cut. August 21, 1912.

Fig. 3.—Field of potatoes in the south field, showing east end of excavation and in the distance the Missouri River. July 8, 1913.

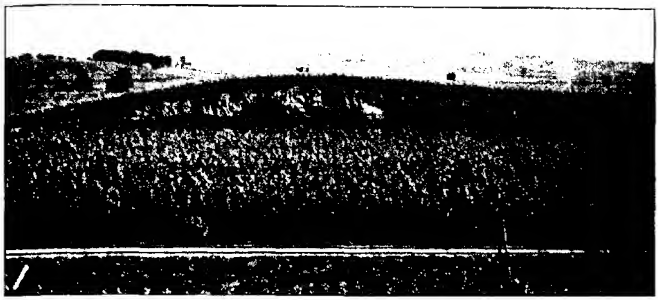

Fig. 1

Fig. 2

Fig. 3

PLATE VIII

Typical views in the two corn fields in the Blair cut on August 21, 1912.
Fig. 1.—In the south field where alfalfa had been grown for 13 years.
Fig. 2.—In the north field where no alfalfa had been grown.

PLATE IX

The fields in the Blair Cut as they appeared on July 8, 1913

Fig. 1.—View from north wall, showing alfalfa in the right foreground, oats with alfalfa in the left foreground, and sweet clover along the railroad track in the middle distance. Between the last and the south wall is a field of potatoes.

Fig. 2.—View from the south wall showing north wall in the distance.

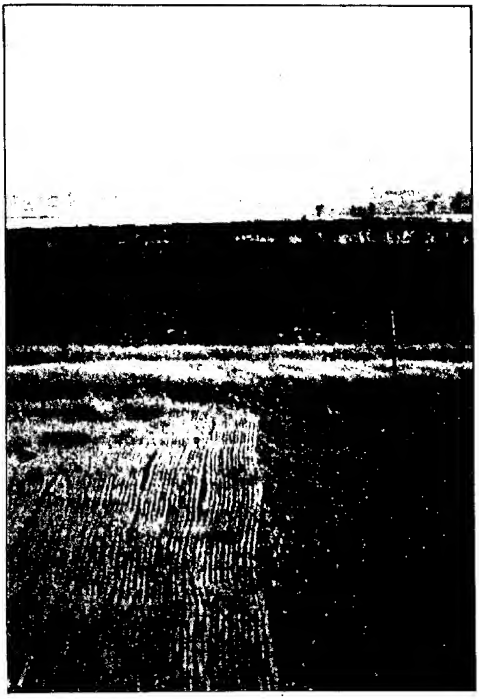

Fig. 1

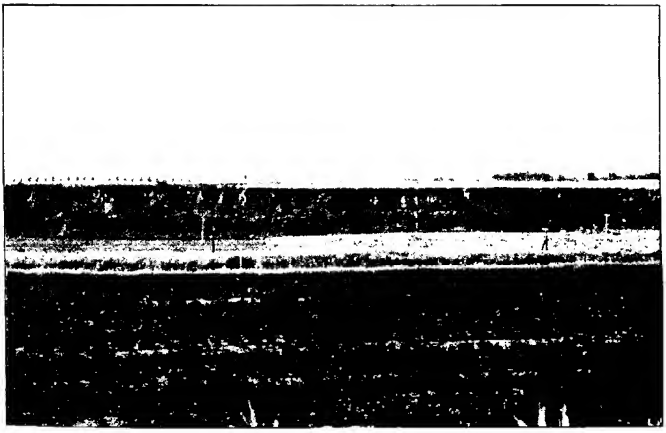

Fig. 2

BIOLOGICAL CHANGES IN SOIL DURING STORAGE[1]

By

F. E. ALLISON,[2] *Research Fellow, Rutgers College*

INTRODUCTION

In soil bacteriological work it is frequently necessary to obtain fresh soil samples from the field for use in the laboratory. It is the common practice to use these soil samples immediately on bringing them into the laboratory or at least within a few hours. This is considered necessary wherever the results obtained are to be a fair indication of the activity or number of organisms in the soil under field conditions. Regardless of the method of sampling used, the soil is always disturbed to a certain extent as in Brown's (1) or any similar method. This disturbing of the soil increases aeration which in turn causes many of the soil organisms to grow much more rapidly. Perhaps some forms are killed off or at least hindered in their development. The work reported below was intended as a study of this particular point. It was planned to learn, if possible, just how long fresh soil samples may be allowed to remain in the laboratory without being appreciably altered in their biological properties. The work is arranged in two parts similar, except that in the first case the soil samples were taken in the winter, and in the second case in the summer. In general, the work consisted of a study of the bacterial changes from the standpoint of numbers and physiological activity. The methods used and the details of procedure are given as each different process is considered.

SOILS USED

Four soils from the college farm were used, the selection being based on fertility, texture and previous cropping systems. They are classed as Penn shaley loam, Penn gravelly loam, Sassafras sandy loam and Alloway clay. The Penn shaley loam was practically neutral in reaction and high in fertility, having been in alfalfa during the preceding year. The Penn gravelly loam was similar to the shaley loam, except that it was slightly more acid. It had grown garden crops for several years. The Sassafras sandy loam was an ordinary farm soil having grown corn, wheat and other general farm crops for several years. It was light in texture, somewhat acid, and of rather low fertility. The Alloway clay was a very heavy, sticky clay of medium fertility, slightly acid and not very well drained. It was in timothy sod when the samples were taken.

[1] Received for publication November 10, 1916.

[2] The writer is indebted to Dr. J. G. Lipman for originally suggesting the problem and for valuable advice during the progress of the work.

PART I

WINTER STUDIES

The date selected for taking the soil samples was February 8, 1916, at a time when the soil was frozen to a depth of about one-half inch and

TABLE I
NUMBERS OF BACTERIA IN SOIL SAMPLED IN WINTER

Time after Sampling	Alloway Clay		Sass. Sandy Loam		Penn Gravelly Loam		Penn Shaley Loam	
	Millions per gm.	Average	Millions per gm.	Average	Millions per gm.	Average	Millions per gm.	Average
0-30 min....	7.9		9.8		6.0		14.0	
	7.4		8.2		5.7		13.5	
	6.3		12.3		5.0		12.0	
	7.0	7.15	8.8	9.77	5.2	5.48	11.5	12.75
2 hours....	4.6		5.3		5.9		9.4	
	4.1		5.5		4.9		8.8	
	4.3		4.7		6.0		8.6	
	4.2	4.30	6.0	5.37	5.8	5.65	6.5	8.33
4 hours....	3.4		4.5		7.8		11.9	
	3.4		5.6		4.3		14.0	
	3.5		5.9		5.9		12.5	
	3.4	3.43	5.6	5.40	4.6	5.65	13.2	12.90
6 hours....	5.5		7.4		5.9		17.5	
	4.4		5.7		6.3		17.5	
	4.1		7.1		5.5		18.7	
	5.0	4.75	7.6	6.95	7.3	6.25	15.0	17.17
10 hours....	3.6		6.9		5.0		13.8	
	4.1		5.8		4.7		14.5	
	3.6		5.7		5.6		13.4	
	5.4	4.18	6.5	6.22	5.0	5.08	15.5	14.30
1 day.....	7.2		9.9		8.7		16.8	
	7.0		10.2		6.8		17.6	
	6.9		10.2		6.7		15.9	
	6.3	6.85	10.0	10.07	6.7	7.23	18.7	17.25
2 days.....	6.9		5.7		5.1		15.1	
	7.9		6.9		5.4		14.2	
	5.6		5.3		4.2		15.4	
	4.6	6.25	4.8	5.68	4.2	4.73	14.5	14.80
3 days.....	4.6		5.5		6.0		11.0	
	2.8		5.6		6.0		11.4	
	4.0		6.8		5.4		10.8	
	3.5	3.72	4.1	5.50	6.5	5.98	11.5	11.18
4 days.....	2.5		3.5		4.8		7.1	
	2.7		3.9		4.4		8.6	
	2.8		3.2		4.4		8.1	
	3.0	2.75	3.9	3.63	3.8	4.35	8.1	7.98
7 days.....	5.0		4.4		5.3		9.7	
	4.3		3.8		5.4		8.3	
	5.5		4.0		4.6		7.9	
	4.3	4.78	4.0	4.05	4.8	5.03	8.6	8.63
10 days.....	8.1		6.5		4.9		9.1	
	7.1		5.6		4.9		8.0	
	5.7		4.8		4.6		8.8	
	6.8	6.93	4.8	5.43	4.0	4.60	8.2	8.53
18 days.....	7.0		4.2		5.1		13.1	
	8.9		6.4		5.7		13.1	
	8.2		4.4		6.4		13.0	
	9.5	8.40	5.4	5.10	5.6	5.70	12.0	12.80

covered with a thin layer of snow. Up to this time of the season the soil had not been frozen to a depth greater than one inch. It was thought

best not to delay the sampling because of the difficulty of obtaining a representative sample from frozen soil. Moreover, upon thawing in the laboratory the soil may not be in a suitable condition for further use. By using soil just below the frozen area practically the same temperature changes are obtained in the laboratory as if the frozen soil itself were used. At the time of collecting the samples the moisture content in the Alloway clay was slightly above the optimum, but the remaining three soils were in excellent physical condition.

The method of sampling consisted of removing the thin layer of frozen soil from an area about 15 to 20 inches square, thoroughly mixing the soil in that area to a depth of about 4 inches with a sterile spatula and

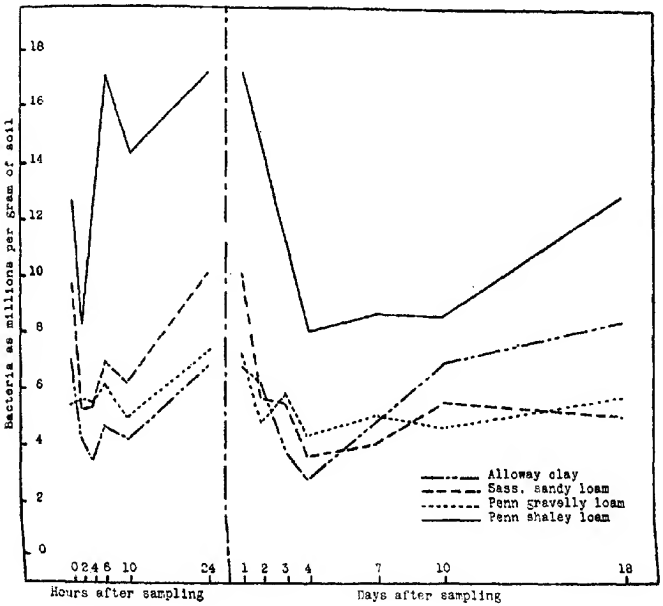

Fig. 1.—Diagram showing numbers of bacteria in soil sampled in winter.

of placing the sample in large sterile glass jars of 5 pounds capacity. The soil was sieved through a coarse sieve as soon as it came into the laboratory, immediately returned to the jars and the latter corked tightly. The first bacteriological tests were started within 30 minutes after bringing the soil into the laboratory.

In Part I numbers of bacteria, ammonification of peptone and dried blood, azofication, and nitrification were determined.

Numbers of Bacteria

For bacterial counts Lipman and Brown's (2) modified synthetic agar was used. A dilution of 1:100,000 was used throughout the work and the plates were incubated at room temperature, 18° to 20° C. for 7 days. Five plates were poured at each sampling for each soil. In numerous cases plates were overrun by rapidly growing fungi and could not be satisfactorily counted. For this reason only the best four, and in some cases the best three plates are given in Table I. These results are shown graphically in figure 1. The broken line in this figure as well as in all the curves which follow separates the results of the first day from those of the later samplings. Necessarily the scale used in plotting is much larger for this first day.

TABLE II

AMMONIFICATION OF PEPTONE BY SOIL SAMPLED IN WINTER

Time after Sampling	Alloway Clay		Sass. Sandy Loam		Penn Gravelly Loam		Penn Shaley Loam	
	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average
0-30 min....	20.0		34.7		30.5		32.4	
	22.5	21.25	40.3	37.50	30.1	30.30	32.6	32.50
2 hours....	14.9		27.7		28.5		25.9	
	14.1	14.50	lost	27.70	30.0	29.25	26.2	26.05
4 hours....	17.2		32.7		32.1		32.3	
	16.5	16.85	32.5	32.60	32.6	32.35	33.9	33.10
6 hours....	23.0		34.6		34.8		38.7	
	22.2	22.60	38.0	36.30	37.5	36.15	39.6	39.15
10 hours....	11.5		22.2		29.3		33.0	
	10.8	11.15	20.1	21.15	31.5	30.40	33.4	33.20
1 day.....	17.2		28.2		30.8		29.6	
	15.2	16.20	32.4	30.30	28.0	29.40	28.7	29.15
2 days.....	16.8		23.0		29.0		26.8	
	18.6	17.70	28.4	25.70	26.3	27.65	27.2	27.00
3 days.....	11.2		20.0		18.6		20.2	
	11.1	11.15	20.5	20.25	21.0	19.80	20.4	20.30
4 days.....	7.4		11.0		11.2		12.6	
	8.1	7.75	11.3	11.15	12.6	11.90	13.6	13.10
7 days.....	4.5		5.8		8.8		9.3	
	4.3	4.40	6.8	6.30	8.6	8.70	10.0	9.65
10 days.....	6.7		7.3		8.0		9.0	
	6.5	6.60	9.6	8.45	9.0	8.50	8.8	8.90
18 days.....	21.5		23.6		27.8		24.9	
	20.4	20.95	28.5	26.05	27.8	27.8	27.2	26.05

The most striking thing brought out in figure 1 is the large increase in the number of bacteria subsequent to the initial decline. No doubt the early increase can be attributed to the rapid rise in temperature when the soil is brought into the warm room. After the first day all soils show a uniform tendency to decrease in numbers until the fourth day and then the trend changes back again until at the end of 18 days from the time of bringing the soil into the laboratory the numbers of bacteria are practically the same as when the soil was first sampled except in the Sassafras sandy loam. It is interesting to note that in general the soils behaved in a similar manner, indicating that change of temperature in the winter,

at least, overbalances all other factors in determining just how the numbers of bacteria will be affected when the soil is removed from its natural condition in the field.

Ammonification of Peptone

The ammonification of peptone was carried out in 250-c.c. Erlenmeyer flasks containing 100 c.c. of a 1 per cent solution of peptone with .05 gm. of K_2HPO_4 per liter. For inoculation 10 c.c. of a 1 to 5 soil infusion was used. After 3 days ammonia was determined by the magnesium oxide method.

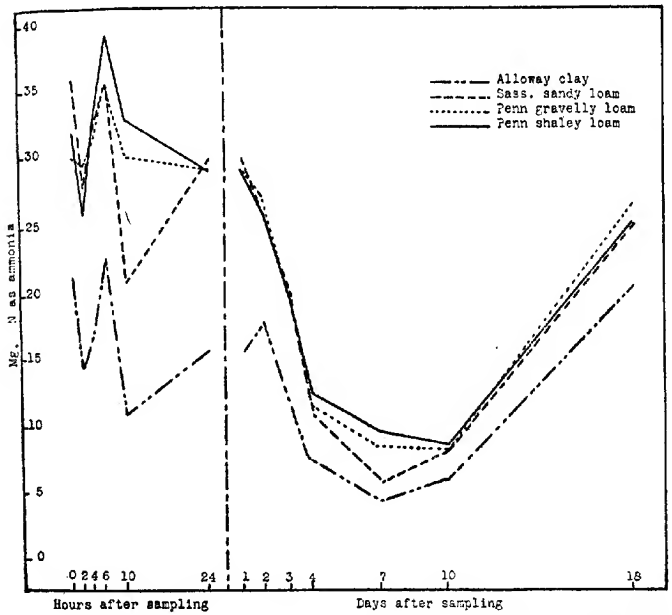

Fig. 2.—Diagram showing ammonification of peptone in solution by soil sampled in winter.

Table II shows clearly that, as was the case with numbers, there seems to be a tendency towards a sudden drop in ammonia production followed by a rise before the end of the first day. After this the ammonia production falls off very rapidly for 3 or 4 days, decreasing in some cases to approximately one-fourth the original ammonifying power. After 10 days all the soils show a very great increase and at the end of 18 days have nearly returned to their original condition as indicated by the amount of ammonia produced. Here, again, and even to a more marked extent than in the case of numbers, the soils tend more and more

to behave in a like manner. By the end of 4 days the differences in ammonifying power of the different soils used had been largely blotted out.

Ammonification of Dried Blood

In order to compare results in solution with those in soil as a medium a series was arranged with sterilized soil. Soils of the plots from which the fresh soil samples were to be taken later were air-dried, 100-gm. portions placed in 250-c.c. Erlenmeyer flasks and thoroughly mixed with dried blood equivalent to 155 mg. N. Water was added but not in quantities sufficient to bring the moisture content up to optimum, thus providing

TABLE III

AMMONIFICATION OF DRIED BLOOD IN SOIL SAMPLED IN WINTER

Time after Sampling	Alloway Clay		Sass. Sandy Loam		Penn Gravelly Loam		Penn Shaley Loam	
	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average
0-30 min....	12.8		14.2		28.6		14.7	
	[1]20.0	12.80	13.4	13.80	25.4	27.00	14.8	14.75
2 hours....	12.7		13.3		21.6		13.9	
	11.2	11.95	13.2	13.25	23.3	22.45	14.9	14.40
4 hours....	12.9		lost		31.3		11.4	
	12.4	12.65	13.5	13.50	25.8	28.55	14.4	12.90
6 hours....	12.3		14.1		29.5		[1]8.0	
	16.2	14.25	15.4	14.75	32.2	30.85	13.4	13.40
10 hours....	14.4		12.5		20.6		12.8	
	10.7	12.55	14.4	13.45	24.8	22.70	14.2	13.50
1 day.....	13.9		12.5		28.0		11.7	
	10.6	12.25	14.4	13.45	22.3	25.15	11.2	11.45
2 days.....	12.6		11.2		23.2		10.8	
	11.2	11.90	12.4	11.80	23.7	23.45	10.2	10.50
3 days.....	6.3		10.6		18.3		9.0	
	6.3	6.30	10.8	10.70	21.3	19.80	9.7	9.35
4 days.....	9.3		10.0		17.0		8.5	
	12.6	10.95	10.4	10.20	16.7	16.85	10.0	9.25
7 days.....	8.5		11.2		16.5		9.4	
	10.0	9.25	9.4	10.30	14.0	15.25	9.1	9.25
10 days.....	lost		13.6		17.2		9.4	
	12.4	12.40	10.7	12.15	16.9	17.05	10.4	9.90
18 days.....	17.0		15.5		27.5		14.3	
	15.7	16.35	14.9	15.20	30.1	28.80	15.9	15.10

[1] Not included in the averages.

against excessive moisture after the addition of the infusion. The flasks were then plugged with cotton and sterilized in the autoclave at 15 pounds pressure for 15 minutes. For inoculation 10 c.c. of a 1 to 5 soil infusion was used for each flask. After 7 days' incubation the ammonia was distilled off with magnesia.

The resemblance in a general way between figures 2 and 3 is a point worthy of note, indicating that the soil bacteria behave similarly whether growing in solution or in their natural medium, the soil. The sterilization of the soil did not change it to so great an extent as to render it undesirable for use in ammonification experiments. When individual soils are considered it will be noted that the tendency of the Sassafras sandy

loam, Alloway clay and Penn gravelly loam is to show a lower ammonifying power, than the Penn shaley loam in peptone solution, but such is not the case in sterilized soil.

Azofication in Solution

For nitrogen fixation 100 c.c. of Winogradski's medium (6) was used. This was inoculated with 10 c.c. of a 1 to 5 soil infusion. At the end of 10 days' incubation at room temperature the contents of the flasks were analyzed for total nitrogen, allowance being made in the table for the nitrogen added in the infusion. The results which are recorded in Table IV are shown graphically in figure 4.

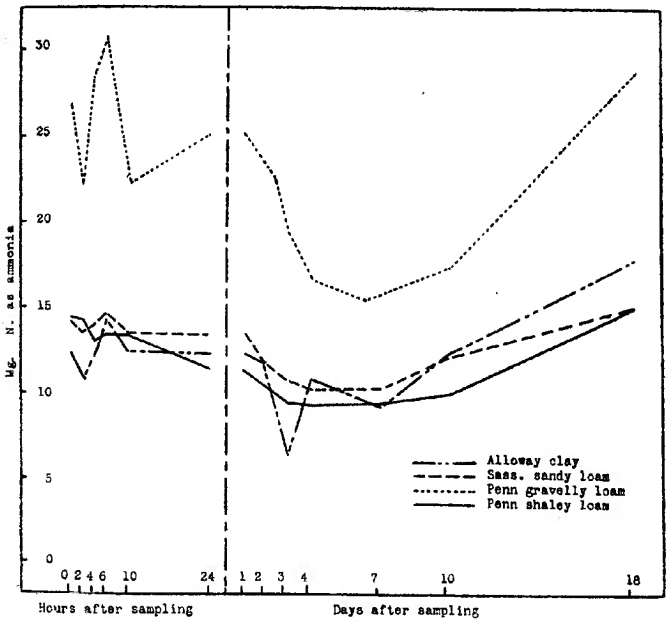

Fig. 3.—Diagram showing ammonification of dried blood in sterilized soil sampled in winter.

The effect of storage on the azofying bacteria of the soils does not seem to be as great as on some of the other groups of organisms present. While in general the trend is towards a decrease in azofying power up to the fourth or seventh day and then an increase, the differences are not great. The fact that the nitrogen-fixing organisms are active, when the soil is very near the freezing point, and capable of fixing fairly large amounts of nitrogen or rapidly become active when conditions are made favorable for their growth is well brought out.

Nitrification in Solution

The medium used was that recommended by Lipman and Brown in their Laboratory Guide in Soil Bacteriology, page 74. It includes among other nutrients, ammonium sulfate at the rate of 1 gm. per 1000 c.c. of water. One hundred-c.c. portions of this medium were placed in 250-c.c. Erlenmeyer flasks, plugged and sterilized in the usual manner. The inoculum consisted of 10 c.c. of a 1 to 5 soil infusion. The incubation period was 2 weeks at room temperature. Table V and figure 5 show the results obtained.

TABLE IV

AZOFICATION IN SOLUTION BY SOIL SAMPLED IN WINTER

Time after Sampling	Alloway Clay		Sass. Sandy Loam		Penn Gravelly Loam		Penn Shaley Loam	
	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average
0-30 min....	6.6		4.6		5.4		6.9	
	9.0	7.80	5.0	4.80	5.3	5.35	6.8	6.85
2 hours....	5.5		lost		3.4		4.3	
	5.2	5.35	3.8	3.80	4.3	3.85	6.1	5.20
4 hours....	5.6		4.0		4.0		8.2	
	lost	5.60	5.6	4.80	4.3	4.15	7.3	7.75
6 hours....	5.9		3.8		3.6		8.4	
	5.8	5.85	3.8	3.80	3.2	3.40	7.9	8.15
10 hours....	5.4		4.1		3.7		8.0	
	5.8	5.60	3.9	4.00	5.0	4.35	8.0	8.00
1 day.....	5.6		4.5		3.5		5.6	
	5.7	5.65	4.3	4.40	3.8	3.65	5.0	5.30
2 days.....	5.7		4.7		2.6		6.5	
	5.7	5.70	4.2	4.45	3.5	3.05	6.9	6.70
3 days.....	5.4		3.8		2.7		6.5	
	4.6	5.00	3.4	3.60	2.7	2.70	6.4	6.45
4 days.....	5.3		3.2		2.2		4.7	
	lost	5.30	3.0	3.10	2.8	2.50	7.4	6.05
7 days.....	5.3		2.8		2.6		6.0	
	5.4	5.35	3.6	3.20	2.9	2.75	6.6	6.30
10 days.....	4.7		3.6		4.1		4.8	
	5.0	4.85	3.9	3.75	3.4	3.75	6.3	5.55
18 days.....	6.4		3.5		3.2		6.9	
	5.8	6.10	3.9	3.70	2.9	3.05	6.7	6.80

In this experiment the mistake was made of not incubating long enough. The time was shortened from the usual 4 weeks because it seemed probable that greater differences would be obtained. Yet at the low temperature of incubation the rate of nitrification was exceedingly slow. However, by referring to figure 5 it will be noted that there are marked differences between the different soils. The Penn shaley loam in most cases shows more than twice as much nitrate as either of the other three soils. By referring to figure 1 it may be seen that numbers and nitrification bear some resemblance to one another in the curves for this particular soil, at least. The amount of nitrate produced in the other three soils was so small that the curves do not have much significance.

Discussion

A general consideration of the above data reveals the fact that soils do change bacteriologically when brought from the field in the winter to the warm laboratory and stored for later use. All of the four representative soils used acted in a somewhat similar manner for each particular physiological process and also as to numbers. The analogy between numbers and these processes, especially ammonification, is also quite marked. This is no doubt due to the fact that bacteria are largely re-

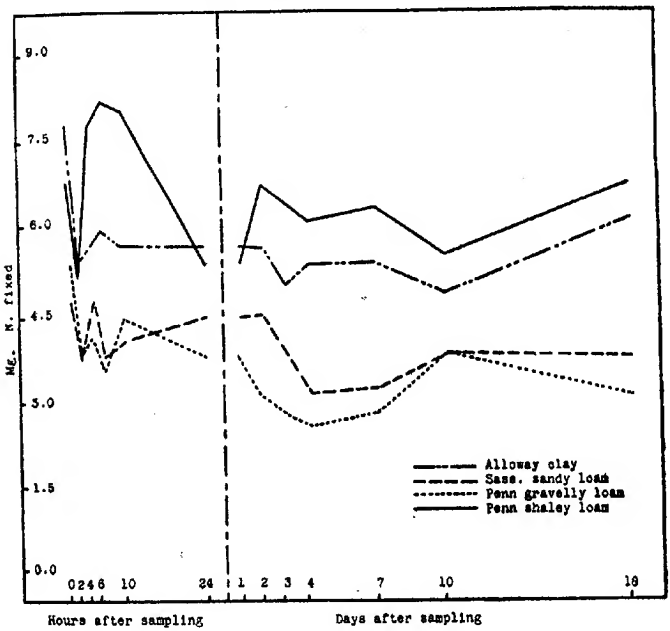

Fig. 4.—Diagram showing azofication in solution by soil sampled in winter.

sponsible for the production of ammonia from dried blood and peptone, and consequently, as the numbers decreased the ammonia production would be expected to show a decrease, provided that there is no serious disturbance of the species relationship. On the other hand, if cottonseed meal had been used there would probably not have been such a close agreement between numbers and ammonia production, since the growth of fungi is favored by the carbohydrates and fats in the cottonseed meal and these organisms would probably consume much of the ammonia produced.

As the primary aim in undertaking this work was to secure data which would show whether soil must be used immediately on being brought into the laboratory if reliable results are to be obtained, it is to be regretted that the question has not been entirely answered. The peculiar results obtained in practically every case on the first day of the experiment are hard to explain. There is almost invariably the sudden drop in the curves until the second hour after the soil was brought into the laboratory followed by almost as abrupt a rise. In some cases this increase continues for 24 hours and in others only until the sixth hour. Probably the bacteria which have become accustomed to low temperatures in the field are injuriously affected by the sudden warming up and aeration of the soil, but after the first shock, are temporarily stimulated.

TABLE V

NITRIFICATION IN SOLUTION BY SOIL SAMPLED IN WINTER

Time after Sampling	Alloway Clay		Sass. Sandy Loam		Penn Gravelly Loam		Penn Shaley Loam	
	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average
0-30 min....	.13		.17		.50		1.45	
	.13	.130	.18	.175	.44	.470	1.11	1.280
2 hours....	.14		.16		.28		0.36	
	.14	.140	.17	.165	.29	.285	0.35	0.355
4 hours....	.15		.17		.25		0.43	
	.15	.150	.17	.170	.24	.245	0.76	0.595
6 hours....	.16		.17		.20		0.37	
	.15	.155	.16	.165	lost	.200	0.39	0.380
10 hours....	.13		.19		.29		0.94	
	.13	.130	.19	.190	.26	.275	0.76	0.850
1 day.....	.15		.19		.24		1.16	
	.17	.160	.19	.190	.29	.265	1.00	1.080
2 days.....	.15		.19		.22		0.52	
	.15	.150	.17	.180	.26	.240	0.63	0.575
3 days.....	.14		.16		.19		0.41	
	.12	.130	.16	.160	.21	.200	0.42	0.415
4 days.....	.12		.15		.17		0.32	
	.14	.130	.14	.145	.18	.175	0.36	0.340
7 days.....	.09		.12		.15		0.42	
	.09	.090	.12	.120	.14	.145	0.40	0.410
10 days.....	.10		.12		.17		0.24	
	.09	.095	.13	.125	.18	.175	0.22	0.230
18 days.....	.09		.10		.13		0.31	
	.08	.085	.11	.105	.17	.150	0.37	0.340

Conn's (2) assumption, that there is a group of warm weather bacteria in the soil and that while one flora is active the other is dormant, may perhaps account for the results reported here. After the temporary increase until the sixth to twenty-fourth hours, a decrease follows because the greater percentage of the bacteria are not favorably influenced by the temperature at which they are kept. To apply Conn's theory still further we must assume that while the cold weather bacteria are dying or at least becoming less active the dormant warm weather organisms are becoming more and more active until by the fourth to tenth days, depending upon the soil and upon the bacteriological process under

consideration, the increase of the one flora overbalances the decrease of the other and the curves start upward again at a rapid rate. At the end of 18 days, the limit of this experiment, the bacterial flora seemed to be just about as active as when first brought from the field.

While Conn's theory seems to explain the phenomena in a reasonable manner it must be remembered that it does not include fungi. Other work done by the writer indicates that the fungi of the soils used directly affected the results, but this is merely a supposition. Perhaps the increase of these organisms at particular times tended to crowd out the

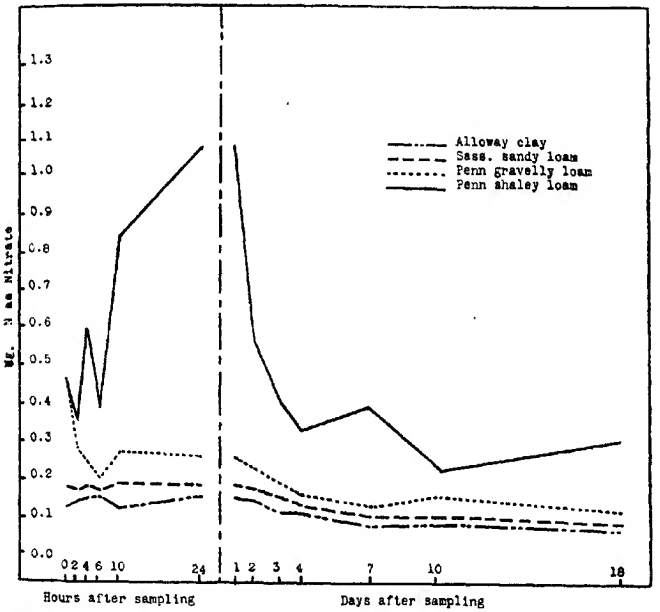

Fig. 5.—Diagram showing nitrification in solution by soil sampled in winter.

bacteria and their decrease at other times allowed a rapid multiplication of the bacteria. It is to be regretted that plates for fungi counts were not poured.

The increase and decrease of bacterial numbers and variations in the extent of chemical changes produced may also be considered from the standpoint of available organic and inorganic nutrients. It will hardly be gainsaid that soil samples when removed from the field and exposed to intense aeration in tumblers are at first likely to accumulate a relatively large amount of available nutrients.

As a whole, the results reported show very strongly that if reliable data are to be obtained, it is necessary to use the soil immediately on be-

ing brought into the laboratory in the winter. A difference of two hours in the case of some of the soils introduced variations of as much as 30 or 40 per cent.

Moist Soil vs. Dry Soil

As a supplement to the studies with moist soil already reported, a few determinations were made with air-dry soil. Portions of each of the

TABLE VI

BACTERIAL EFFECTS OF DRYING SOIL SAMPLES IN WINTER

NUMBER OF BACTERIA

Alloway Clay			Sassafras Sandy Loam			Penn Gravelly Loam			Penn Shaley Loam		
Moist	Air-dry		Moist	Air-dry		Moist	Air-dry		Moist	Air-dry	
Mill'ns per gm.	Mill'ns per gm.	Av'ge	Mill'ns per gm.	Mill'ns per gm.	Av'ge	Mill'ns per gm.	Mill'ns per gm.	Av'ge	Mill'ns per gm.	Mill'ns per gm.	Av'ge
	1.80			1.30			6.20			19.00	
	1.90			1.40			7.20			16.70	
	1.40			2.20			6.10			17.70	
6.85	1.50	1.65	10.07	2.60	1.88	7.23	6.10	6.40	17.25	17.80	17.80
AMMONIFICATION OF PEPTONE											
Mg.N.	Mg.N.	Av'ge	Mg.N.	Mg.N.	Av'ge	Mg.N.	Mg.N.	Av'ge	Mg.N.	Mg.N.	Av'ge
	4.50			8.00			6.00			8.10	
16.20	4.00	4.25	30.30	9.80	8.90	29.40	5.70	5.85	29.25	8.40	8.25
AMMONIFICATION OF DRIED BLOOD											
	12.60			9.40			22.50			7.40	
12.25	12.60	12.60	13.45	10.70	10.05	25.15	24.20	23.35	11.45	8.20	7.80
AZOFICATION IN SOLUTION											
	5.00			3.50			3.20			6.20	
5.65	6.30	5.65	4.40	3.80	3.65	3.65	3.30	3.25	5.30	5.80	6.00
NITRIFICATION IN SOLUTION											
	0.12			0.14			0.17			lost	
0.16	0.14	0.13	0.19	0.15	0.14	0.26	0.22	0.19	1.08	0.44	0.44

four moist soils were removed from the large bottles on the second day after sampling and spread out in the open air of the dark incubator room. After about a week had elapsed plates were poured and flasks inoculated for ammonification, nitrification and azofication as in the case of the moist soil samples. The results obtained are given in Table VI together

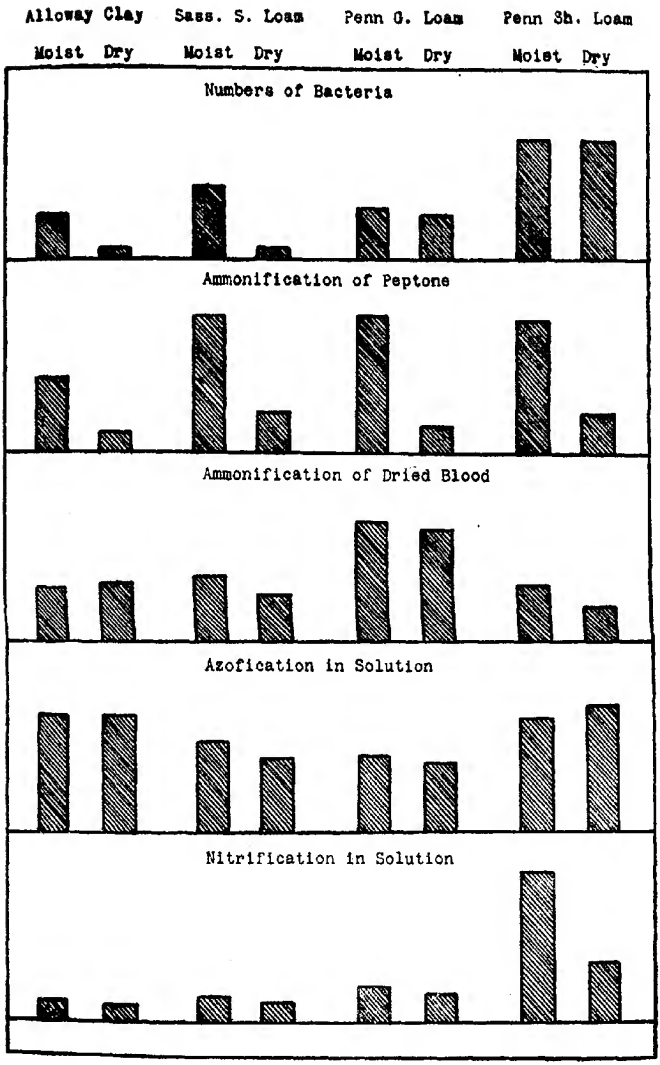

Fig. 6.—Diagram showing bacterial effects of air-drying soil sampled in winter.

with the average results obtained with the moist soil samples for the second day of storage, corresponding with the time when the air-dried samples were first spread out to dry. The results for moist soils are the same as reported in previous tables. In figure 6 the results are shown diagramatically.

Generally, there is a marked decrease in bacterial numbers and activities due to air-drying. In the case of numbers, for instance, the decrease is very striking for the clay and sandy loam soils, only slight for the gravelly loam and entirely absent in the case of the shaley loam. In the ammonification of peptone the decrease is very marked for all soils, but dried blood for some unknown reason was broken down almost as readily by the organisms from the air-dried soil as from the moist soil. Similarly, in azofication the difference between the moist and air-dried soils is so slight as to be entirely within experimental error. As for nitrification little can be said because the amounts of nitrates produced were so small; but the indications point to an injurious effect through air-drying.

PART II

SUMMER STUDIES

After the completion of the work reported in Part I it seemed desirable to repeat the work during the summer months when entirely different temperature conditions prevail. The winter results indicated that the more marked differences obtained were due chiefly to the temperature changes rather than to increased aeration or chemical changes. If such was the case then the change occurring on bringing moist soil into the laboratory should not be great if the temperature change was not marked, and hence it would not be necessary to use soil samples for biological work as soon as brought from the field.

For the work of Part II only two of the four soils previously used were chosen, the Penn shaley loam and the Sassafras sandy loam, two widely different types biologically. At the time of sampling, July 20, 1916, the shale soil was in rye stubble and at as near the physical optimum in moisture as it is possible to determine. The sandy loam soil had been in fallow all summer and was free from all vegetation. It was at the optimum moisture content at the time of sampling.

The method of taking the soil samples was the same as that previously discussed. However, after bringing the samples into the laboratory and sieving, they were divided into halves, one portion being placed in 5-pound glass jars and corked tightly and the other portion into a similar jar and a large cotton plug inserted. This procedure would tend to show whether or not aeration is a factor of importance in the storage of moist soil.

In Part II numbers of bacteria and fungi and ammonification of peptone, dried blood and cottonseed meal were determined.

TABLE VII

NUMBERS OF BACTERIA IN SOIL SAMPLED IN SUMMER

Time after Sampling	Sassafras Sandy Loam				Penn Shaley Loam			
	Stoppered		Cotton Plug		Stoppered		Cotton Plug	
	Millions per gm.	Average	Millions per gm.	Average	Millions per gm.	Average	Millions per gm.	Average
0-30 min....	6.4				18.3			
	6.0				19.3			
	6.1				17.1			
	6.6	6.28			15.4	17.53		
2 hours....	6.0				14.8			
	7.5				16.0			
	4.5				19.0			
	6.2	6.05			fungi	16.60		
4 hours....	5.9				13.7			
	6.1				13.5			
	6.4				16.5			
	5.6	6.00			fungi	14.57		
8 hours....	6.2				11.8			
	5.7				14.7			
	8.2				12.9			
	6.8	6.73			13.9	13.33		
1 day.....	12.8		12.5		12.4		13.0	
	11.6		13.5		12.5		14.6	
	10.1		11.0		15.7		14.9	
	fungi	11.50	12.9	12.48	14.8	13.85	16.1	14.65
2 days.....	6.9		8.7		13.9		14.5	
	6.0		6.6		18.2		16.5	
	6.2		9.2		17.0		15.2	
	fungi	6.33	9.6	8.53	14.6	15.93	15.5	15.43
3 days.....	7.2		9.3		14.8		18.3	
	6.8		7.2		17.4		17.2	
	fungi		9.5		16.2		18.1	
	6.5	6.83	9.1	8.78	15.1	15.89	17.8	17.85
10 days.....	5.3		8.1		18.0		15.3	
	7.2		9.1		18.6		18.7	
	7.6		10.0		16.9		18.2	
	6.0	6.53	8.0	8.80	16.1	17.40	14.7	16.73
20 days.....	5.5		5.9		12.7		11.5	
	5.8		6.4		12.8		16.0	
	5.9		5.6		14.0		12.4	
	5.8	5.75	6.2	6.03	16.1	13.90	10.0	12.47
30 days.....	2.8		2.5		10.5		8.4	
	2.4		4.1		11.6		8.4	
	2.3		3.1		10.9		7.8	
	2.5	2.50	2.0	2.93	11.9	11.23	7.5	8.03
40 days.....	fungi		1.3		4.7		3.0	
	1.3		1.0		4.2		2.9	
	2.1		1.5		3.0		3.8	
	2.3	1.90	1.7	1.38	3.7	3.90	3.5	3.30
60 days.....	2.9		2.6		2.9		2.4	
	2.0		2.1		3.4		2.1	
	1.8		3.0		2.9		2.5	
	fungi	2.23	1.8	2.38	2.6	2.95	2.2	2.30

Numbers of Bacteria

Five plates of a 1:100,000 dilution were poured, Lipman and Brown's (5) modified synthetic agar being used. The best four counts are given in the table, as was done in the preceding work. Incubation was at room temperature, about 23° C. The individual results are given in Table VII and the graph in figure 7.

For the first 20 days it will be noticed that there was no marked decrease in bacterial numbers as compared with the first sampling in either the sand or loam soils. There were some increases, though, particularly

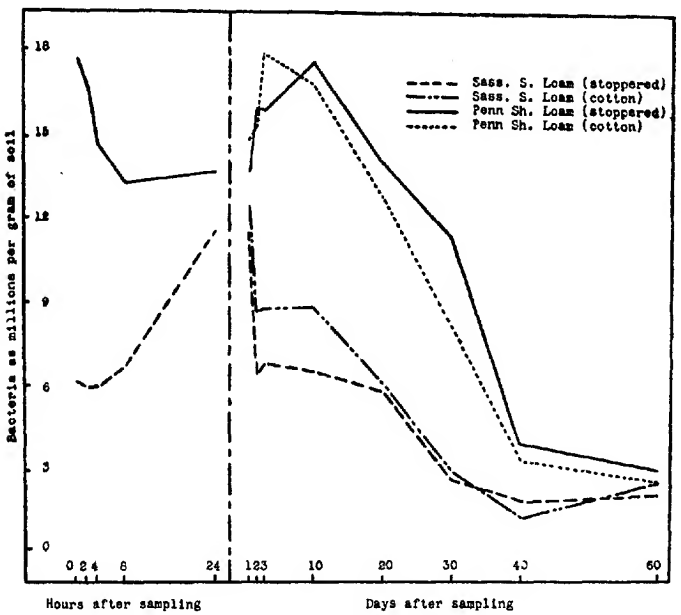

Fig. 7.—Diagram showing numbers of bacteria in soil sampled in summer.

in the sandy soil where at the end of 24 hours the number of colonies developing on the plates had nearly doubled. This increase was followed by a sudden decrease for two or three days and then a more gradual diminution until at the end of the 40-day period less than one-third as many colonies were present as at first. The shale shows similar results except that there was a decrease for the first few hours and then only a slight increase as compared with the larger increase in the sandy soil.

The marked similarity of the curves for the bottles tightly corked and for those with cotton plugs is significant. In both soils the differences are easily within experimental error, showing either that lack of aeration in the bottles is not the cause of the decrease in numbers or that the cotton plug does not permit enough air circulation to meet the needs of the organisms. Moisture determinations of the soil were made at the

TABLE VIII

NUMBERS OF FUNGI IN SOIL SAMPLED IN SUMMER

Time after Sampling	Sassafras Sandy Loam				Penn Shaley Loam			
	Stoppered		Cotton Plug		Stoppered		Cotton Plug	
	Thous'ds per gm.	Average	Thous'ds per gm.	Average	Thous'ds per gm.	Average	Thous'ds per gm.	Average
0-30 min....	130				130			
	130				120			
	150				100			
	100	127.5			120	117.5		
2 hours....	120				130			
	110				130			
	100				200			
	100	107.5			170	157.5		
4 hours....	180				170			
	180				190			
	150				180			
	170	170.0			150	172.5		
8 hours....	120				160			
	130				lost			
	140				140			
	120	127.5			110	136.6		
1 day.....	90		100		140		160	
	90		100		150		170	
	80		90		160		170	
	70	82.5	100	97.5	150	150.0	110	160.0
2 days.....	130		lost		160		170	
	110		100		190		170	
	160		110		220		150	
	120	130.0	120	110.0	220	197.5	140	157.5
3 days.....	160		120		230		190	
	120		130		160		240	
	120		130		220		220	
	120	130.0	110	122.5	200	202.5	260	227.5
5 days.....	90		120		130		190	
	100		lost		110		200	
	80		100		120		190	
	80	87.5	80	100.0	120	120.0	210	197.5
10 days.....	110		120		160		200	
	90		90		170		200	
	80		100		200		260	
	80	90.0	110	105.0	210	185.0	260	230.0
20 days.....	120		100		220		150	
	lost		110		150		180	
	160		90		160		150	
	110	130.0	100	100.0	200	182.5	150	157.5
30 days.....	150		110		180		220	
	140		110		210		220	
	150		90		240		210	
	170	152.5	120	107.5	220	212.5	250	225.0
40 days.....	170		lost		160		170	
	160		110		140		210	
	190		130		180		240	
	170	172.5	120	120.0	180	165.0	190	202.5
60 days.....	140		90		180		310	
	140		100		160		250	
	150		110		180		270	
	140	142.5	100	100.0	180	175.0	270	275.0

beginning and at the end of the experiment, but the loss in the cotton-plugged bottles was only 0.2 per cent for the sand and 0.3 per cent for the loam. The bottles had been kept in a moist, dark room to insure against serious loss by evaporation.

A comparison of the winter and summer results shows some similarity for the first few days, but the decrease in numbers is much more rapid in the winter. The big increase noticed during the winter after the

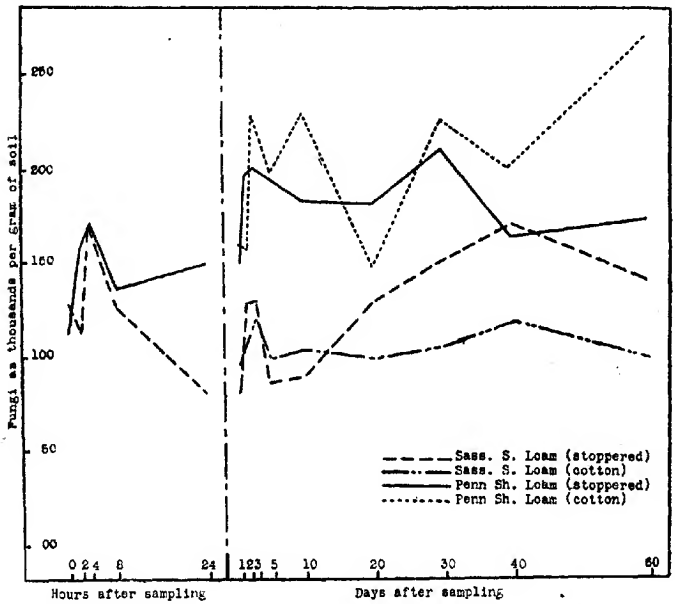

Fig. 8.—Diagram showing numbers of fungi, in soil sampled in summer.

first few days does not appear in the summer. After 60 days there was a slight increase in numbers in the sandy loam, but the decrease still continued in the heavier soil. Perhaps if the experiment had been continued longer there would have been marked increases.

Numbers of Fungi

The medium used for fungi was Cook and Taubenhaus' (3) No. 2. A dilution of 1:10,000 was used throughout the work, the plates incubated at room temperature and counted at the end of 4 days. Quite often a plate would be overrun by a rapidly growing fungus so that the count of only the best four plates are given in Table VIII. Figure 8 shows the results graphically.

Contrary to the results obtained for bacterial numbers the fungi seem to increase during storage or at least do not show any decided decreases.

The curve for the loam soil goes up and down frequently and so no very definite conclusions can be drawn. The tendency is toward an increase rather than a decrease. The sand shows a diminution for the first few days and then a gradual increase. After 40 days the curve drops slightly.

The differences between the tightly corked bottles and those with cotton plugs are somewhat greater here than in the case of bacteria, but not very significant since the behavior is different for the soils. The use of cotton plugs allowed a greater increase in the number of fungi in the loam and caused a decrease in the sand.

TABLE IX

AMMONIFICATION OF PEPTONE BY SOIL SAMPLED IN SUMMER

Time after Sampling	Sassafras Sandy Loam				Penn Shaley Loam			
	Stoppered		Cotton Plug		Stoppered		Cotton Plug	
	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average
0-30 min....	58.3				58.3			
	61.6	59.95			54.2	56.25		
2 hours....	61.7				54.0			
	60.6	61.15			55.6	54.80		
4 hours....	48.8				54.9			
	54.4	51.60			58.8	56.85		
8 hours....	53.3				42.7			
	48.6	50.95			49.9	46.30		
1 day.....	57.2		51.9		52.2		56.0	
	55.3	56.25	58.6	55.25	52.9	52.55	54.8	55.40
2 days.....	52.7		57.8		47.6		54.1	
	49.9	51.30	52.8	55.30	48.2	47.90	48.8	51.45
3 days.....	47.1		45.1		47.3		46.7	
	52.3	49.70	48.2	46.65	44.8	46.05	40.2	43.45
5 days.....	51.3		52.0		50.8		50.8	
	55.9	53.60	52.5	52.25	50.5	50.65	49.2	50.00
10 days.....	53.0		55.0		51.4		57.1	
	52.9	52.95	53.6	54.30	51.7	51.55	53.8	55.45
20 days.....	49.4		50.2		45.7		46.2	
	58.0	53.70	48.7	49.45	43.3	44.50	45.7	45.95
30 days.....	48.1		55.9		46.6		47.2	
	47.6	47.85	45.8	50.85	46.8	46.70	44.4	45.80
40 days.....	45.0		42.9		39.6		41.6	
	41.3	43.15	39.8	41.35	41.5	40.55	38.7	40.15
60 days.....	24.5		22.5		22.0		16.0	
	16.5	20.50	lost	22.50	16.7	19.35	16.2	16.10

Ammonification of Peptone

The method used for the ammonification of peptone was the same as that discussed in Part I except that only 5 c.c. of a 1 to 5 soil infusion was used for inoculation. The results are shown in Table IX and figure 9.

In general, it will be noticed that there is a similarity between these curves and the bacterial-number curves in figure 7, especially at the later dates. The fluctuations are not as great at first in the peptone curve, but after about 10 days the constant drop is quite significant. It indicates very strongly that bacteria and not fungi are primarily responsible for

the breaking down of peptone. Perhaps if fungi were entirely absent the number curves and the peptone curves would agree more closely.

The use of cotton plugs did not lessen the decrease in bacterial activities, this being in agreement with the results obtained for numbers.

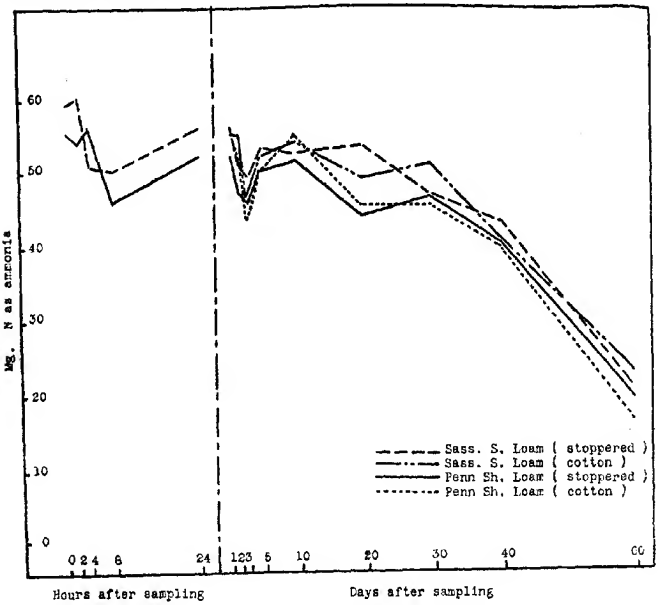

Fig. 9.—Diagram showing ammonification of peptone in solution by soil sampled in summer.

Ammonification of Dried Blood

The fresh-soil-tumbler method was employed, 100 gm. of moist soil and the equivalent of 155 mg. of nitrogen as dried blood being used. The mixing was done by means of the soil shaker described by Lint and Coleman (4) and the incubation carried on at room temperature for 6 days. The results are given in Table X and shown graphically in figure 10.

The similarity between the ammonification of dried blood and that of peptone shows that dried blood also is largely broken down by bacteria rather than by fungi, but since the diminution after 20 to 40 days is not as great, it seems very probable that fungi play a more prominent part in the ammonification of dried blood than of peptone. Again the use of cotton plugs did not change the results.

Ammonification of Cottonseed Meal

The procedure here was the same as that for dried blood, except for the different source of organic matter employed. No determinations were made

with soil from the bottles containing cotton plugs. Table XI and the corresponding figure 11, give the results obtained.

The results throughout the 60-day period were much alike. The slight increases and decreases are not significant. While there is no definite agreement between the amount of ammonia produced and the numbers of fungi there is, nevertheless, enough similarity to show that fungi are a more important factor in the breaking down of cottonseed meal than are bacteria, contrary to the results obtained with dried blood. The balance between the decrease in bacterial activities and the increase in those of the fungi seems to have kept the ammonification results fairly constant.

TABLE X

AMMONIFICATION OF DRIED BLOOD BY SOIL SAMPLED IN SUMMER

Time after Sampling	Sassafras Sandy Loam				Penn Shaley Loam			
	Stoppered		Cotton Plug		Stoppered		Cotton Plug	
	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average	Mg. N.	Average
0-30 min....	33.5				27.4			
	33.0	33.25			25.3	26.35		
2 hours....	31.4				25.6			
	33.7	32.55			25.7	25.65		
4 hours....	32.6				24.3			
	33.9	33.25			25.4	24.85		
8 hours....	31.8				24.5			
	32.9	32.35			24.9	24.70		
1 day.....	35.4		32.6		24.3		23.4	
	35.1	35.25	33.6	33.10	25.4	24.85	26.1	24.75
2 days.....	lost		32.4		22.7		22.1	
	32.0	32.00	32.6	32.5	23.2	22.95	21.7	21.90
3 days.....	32.3		33.7		22.2		lost	
	33.5	32.90	33.4	33.55	22.7	22.45	25.9	25.90
5 days.....	34.5		33.8		lost		22.2	
	34.3	34.40	34.0	33.90	23.3	23.30	22.7	22.45
10 days.....	35.2		35.5		22.6		21.1	
	37.8	36.50	30.9	33.20	20.8	21.70	23.9	22.50
20 days.....	32.9		35.7		20.7		19.3	
	35.5	34.20	37.2	36.45	19.0	19.85	22.4	20.85
30 days.....	32.3		33.8		20.3		20.2	
	33.1	32.70	34.1	33.95	21.5	20.9	20.5	20.35
40 days.....	25.7		27.3		19.6		16.5	
	26.5	26.10	27.2	27.25	18.9	19.25	21.8	19.15
60 days.....	23.0		23.6		13.8		14.3	
	22.1	22.55	22.2	22.90	14.7	14.25	14.8	14.55

Discussion

The summer work, while to some extent agreeing with the winter results, shows that biologically the soil changes much less abruptly during the warmer weather. The variations during the first day in numbers of fungi and bacteria were in some cases sufficiently marked to show that the sooner the sample is used after being brought from the field, the more reliable the data will be. The differences in the case of ammonification were very slight and fully within the experimental error. It is not

until the tenth to twentieth day that the pronounced changes begin to take place. The numbers of bacteria and the ammonification of peptone and dried blood begin to decrease rather uniformly, while the number of fungi shows a tendency to remain at the same level or to increase slightly. As has already been brought out, the ammonia production from cotton-seed meal may depend to a marked degree on fungi and hence shows a tendency to remain fairly constant.

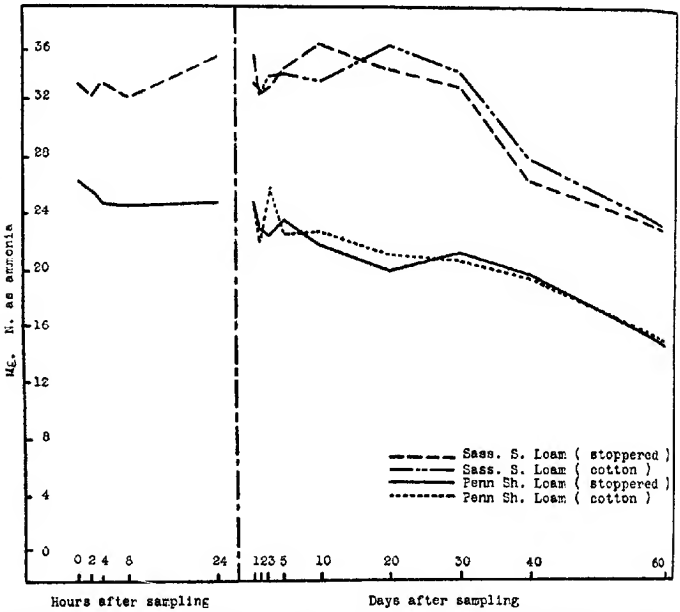

Fig. 10.—Diagram showing ammonification of dried blood in fresh soil sampled in summer.

The explanation of the summer results probably lies in the method of storing the soil rather than in the mere fact that the soil was transferred from the field to the laboratory. In the 5-pound bottles used there is without doubt a limited circulation of the air even where the cotton plugs were used. The oxygen supply is probably limited and the accumulation of substances more or less toxic to the organisms increases. The air in the containers while not directly detrimental may hinder rapid growth because of the lessened supply of oxygen. The increases in fungi and also in bacteria, except in the shaley loam, during the first few hours after sampling, bears out the common belief that stirring of the soil causes a sudden increase in the number of soil organisms, provided that moisture and other environmental conditions are not limiting factors. Following this first increase there is a rapid return to normal and a ten-

dency to continue the same for several days. After the decrease in numbers and physiological efficiency once begins we should not expect another increase, provided lack of oxygen is the cause of the decrease, unless a flora of anaerobic organisms should develop.

From the standpoint of soil biological methods the work reported in Part I and Part II shows that soil samples taken in the winter should without question be used immediately after sampling, especially if numbers are under consideration. In the summer it is desirable to follow the same practice, though this is less essential. All plates for counts should be poured soon after sampling, but there is no great necessity for haste in starting ammonification experiments.

TABLE XI

AMMONIFICATION OF COTTONSEED MEAL BY SOIL SAMPLED IN SUMMER

Time after Sampling	Sassafras Sandy Loam		Penn Shaley Loam	
	Mg. N.	Average	Mg. N.	Average
0-30 min.	70.9		59.6	
	72.9	71.90	[1]71.8	59.60
2 hours	[1]60.9		60.1	
	73.7	73.70	61.1	60.55
4 hours	74.8		58.0	
	71.2	73.00	58.9	58.45
8 hours	70.6		59.2	
	69.6	70.10	60.5	59.85
1 day	74.2		61.4	
	71.6	72.90	61.5	61.45
2 days	73.5		59.4	
	73.8	73.65	59.4	59.40
3 days	73.5		60.7	
	73.6	73.55	60.7	60.70
5 days	72.8		57.1	
	70.9	71.85	55.6	56.35
10 days	73.7		61.1	
	69.0	71.35	57.5	59.30
20 days	70.9		57.3	
	72.0	71.45	59.3	58.30
30 days	[1]85.3		61.8	
	77.1	77.10	66.7	64.25
40 days	71.5		60.3	
	73.2	72.35	56.8	58.55
60 days	71.5		54.4	
	73.4	72.45	56.5	55.45

[1] Not included in the averages.

Moist Soil vs. Dry Soil

The effect of air-drying soil on bacterial numbers in the summer was also studied on a small scale as a supplement to Part II. In this case some of the soil was spread out to dry in the open air of the dark incubating room as soon as the sample was brought in from the field. After one month had elapsed the numbers were determined and the ammonification experiments started. The results obtained are given in Table XII together with the average results for the moist soil previously given. Figure 12 was drawn from this data.

The decrease in numbers of bacteria on air-drying was very marked in both of the soils. As will be remembered, the winter results showed a similar decrease for the Sassafras soil, but no decrease in the case of the Penn loam. Drying caused some decrease in numbers of fungi, especially in the sandy soil, but not nearly to as great an extent as was found with bacteria.

TABLE XII

BIOLOGICAL EFFECTS OF DRYING SOIL SAMPLED IN SUMMER

NUMBERS OF BACTERIA

Sassafras Sandy Loam			Penn Shaley Loam		
Moist	Air-dry		Moist	Air-dry	
Millions per gm.	Millions per gm.	Average	Millions per gm.	Millions per gm.	Average
	1.60			1.70	
	1.10			2.40	
	1.80			1.50	
6.28	1.30	1.45	16.64	fungi	1.87

NUMBERS OF FUNGI

Thousands per gm.	Thousands per gm.	Average	Thousands per gm.	Thousands per gm.	Average
	50			120	
	50			80	
	90			80	
127.5	fungi	63.30	112.50	110	97.5

AMMONIFICATION OF PEPTONE

Mg. N	Mg. N.	Average	Mg. N.	Mg. N.	Average
	48.20			31.10	
59.95	46.80	47.50	56.25	30.30	30.70

AMMONIFICATION OF DRIED BLOOD

	32.50			36.60	
33.25	30.40	31.45	26.35	33.50	35.05

AMMONIFICATION OF COTTONSEED MEAL

	65.20			61.06	
71.90	67.70	66.45	59.60	lost	61.06

The ammonification results as a whole show little change due to drying with the possible exception of the peptone determinations. Here, as was the case in the winter, but not to as great an extent, the ammonia production decreased more in the solution work than where soil was used as a medium with dried blood as the source of nitrogen. Indeed the summer results show an increase in ammonification through air-drying the Penn loam soil. Cottonseed meal gave results very similar to those of dried blood, there being little or no decrease due to air-drying.

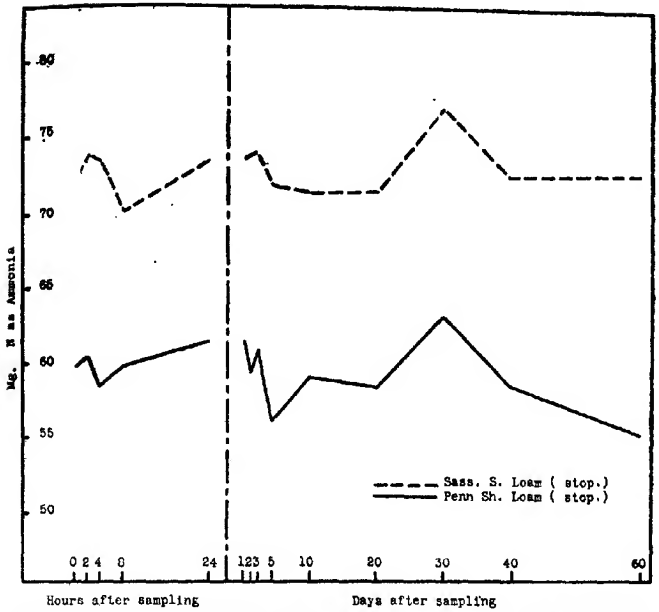

Fig. 11.—Diagram showing ammonification of cottonseed meal in fresh soil sampled in summer.

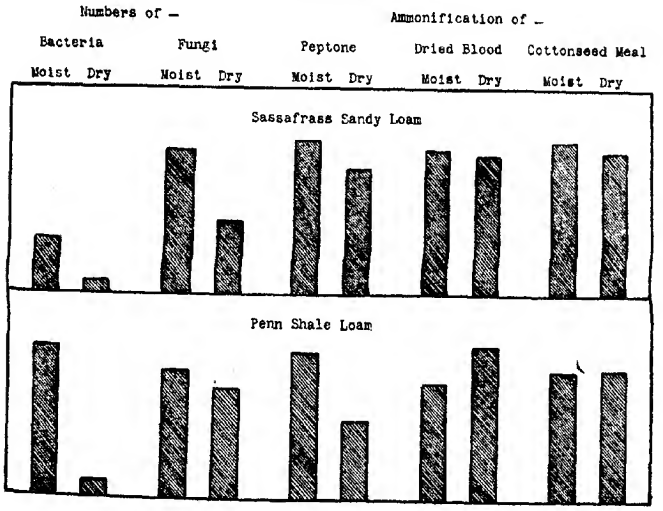

Fig. 12.—Diagram showing biological effects of air-drying soil sampled in summer.

SUMMARY

1. Soils change biologically to a very marked extent during storage in the laboratory, and the rate of change depends largely upon the temperature of the soil when sampled. A change in numbers of bacteria in 2 hours amounted to as much as 30 to 40 per cent in some soils during the winter months.

2. There is a decided tendency for the ammonification of dried blood and peptone to vary as the numbers of bacteria vary; and for the ammonia production from cottonseed meal to go hand in hand with the numbers of fungi.

3. In the winter there is a diminution in bacterial numbers and usually in ammonification until the end of one week and this is followed by a steady increase. In the summer the decrease in numbers and ammonia production from peptone and dried blood proceeds more slowly and continues for at least 2 months. To the very end of this experiment the point had not been reached where ammonification ceased to decrease.

4. From the data presented in this paper it may be said that in order to obtain reliable results during the winter months it is necessary to pour plates for bacterial counts and start all ammonification experiments immediately on bringing the soil sample into the warm laboratory. During the summer months it is desirable to pour plates soon after taking the sample, but this is not as essential as during the colder months. Ammonification experiments need not be started with such haste. During the first ten days the variations in ammonification were almost within the limits of experimental error.

5. Air-drying caused a decided decrease in numbers of bacteria except in the case of the Penn loam during the winter months. The decrease was much less marked for fungi than for bacteria. Air-drying had very little effect on the ammonification of dried blood and cottonseed meal, but caused a pronounced diminution in ammonia production from peptone in solution. Nitrogen-fixation results were very little affected.

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AMMONIFIABILITY VERSUS NITRIFIABILITY AS A TEST FOR THE RELATIVE AVAILABILITY OF NITROGENOUS FERTILIZERS[1]

By

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INTRODUCTORY

The onerous, costly, and time consuming features of the method of determining the availability of nitrogen in different fertilizers by vegetation tests have long since caused soil and agricultural chemists to attempt to find a simpler one. To such attempts we owe such purely chemical methods as the alkaline permanganate method[1] and such bacterio-chemical methods as J. G. Lipman's (6) ammonifiability coefficient. The first named is purely arbitrary, and while some vegetation tests have confirmed its partial validity, it is still very faulty owing in large part to the great diversity in the constitution of different soils. The second one, while also arbitrary, is less so since it is known that nitrogen in all forms but those of ammonia, nitrite and nitrate, is more or less rapidly transformed into ammonia in the soil. In both methods the assumption is made that oxidation of organic nitrogen, or its transformation into ammonia is evidence of the fact that that nitrogen will also be transformed at a similar, or sufficiently rapid rate, into nitrate. This is so assumed manifestly for the reason that ammonia is merely a transition stage in the cycle of bacterial transformation of organic nitrogen into nitrate. Indeed, the assumption just referred to has been made in all of the recent investigations dealing with ammonification in soils and has been chiefly responsible for the large number of ammonification tests which have been carried out with fertilizers. A minor reason for the widespread use of the ammonifiability or the ammonification coefficient methods has of course been the simplicity thereof coupled with the short period of incubation required.

For several years past the senior author has suspected that the assumption above cited is not always substantiated by the facts. Our recent investigations on the subject carried out in connection with some other researches prove the suspicion to be well founded. It is now our purpose to describe very briefly our experiments. On the basis that nitrates con-

[1] Received for publication September 26, 1916.

stitute the only stable form in the soil of the simple transformation products of organic nitrogen and on the assumption that plants, taken by and large, prefer nitrates to other forms of nitrogen, we decided to establish nitrifiability instead of ammonifiability as a criterion for the availability of fertilizers. Despite the longer incubation period and more difficult analytical technique involved, the nitrifiability method appears to be entirely feasible. The results obtained with it in the study of nearly thirty soil types with a large variety of fertilizers, have been detailed elsewhere (4). Along with the work on nitrifiability, however, we also carried out corresponding ammonifiability tests with the same fertilizers, and in the same soil types. The results of these further tests have not yet been reported, and we are therefore making them the principal subject of discussion in this paper. For the sake of more easy comparison of the ammonifiability with the nitrifiability data, the latter are repeated from the publication above cited and included in the tables accompanying the present discussion.

Methods Employed

The usual technique now so largely employed in soils laboratories was applied to our studies. Briefly, the ammonification tests were run by the tumbler method, 1 gm. of the fertilizer being mixed with 50 gm. of the soil in the case of all fertilizers and all soils. The incubation period was 7 days and the temperature 28° to 30° C. As nearly as possible optimum moisture conditions were supplied. At the end of the incubation period ammonia was determined by the magnesia-distillation method. In the nitrification work with which the present ammonification data are here compared, 100-gm. samples of soil were employed and only 1 per cent of the nitrogenous fertilizer. The incubation period was 4 weeks at the same temperature as that given above. Nitrates were determined by the modified form of colorimetric method (5) in vogue in this laboratory for the past five years. The data obtained are so arranged in the tables that a given nitrogenous material both as to ammonifiability and as to nitrifiability may be studied in the same table in all the soils used. Furthermore, every table gives for both the ammonification and nitrification data a statement of the number of milligrams of ammonia nitrogen produced in duplicate cultures, the average ammonia production, and the percentage of the total nitrogen ammonified. Similar data are given for the nitrification tests. In the case of ammonification, the total nitrogen present included only that in the fertilizer used, while the total nitrogen values used in the nitrification data included soil and fertilizer nitrogen.

Discussion of the Results

Tables I, II, III, IV and V furnish a detailed statement of the data referred to in the foregoing paragraph. Because of the arrangement em-

TABLE I

AMMONIFICATION AND NITRIFICATION OF DRIED BLOOD

No.	Soil	Ammonification			Nitrification		
		Mg. N Over Blank	Average	% Made Available	Mg. N Nitrified	Average	% N Available
I	Anaheim	38.85	38.99	31.69	4.72	4.92	2.6
		39.13			5.12		
II	Oakley sandy	26.18	25.83	21.00	0.00	.....	.....
		25.48			0.00		
III	Covina sandy	44.31	44.87	36.48	3.72	3.57	1.9
		45.43			3.42		
IV	Pomona sandy loam	45.22	47.18	38.35	1.77	1.77	0.7
		49.14			1.77		
V	Watsonville strawberry soil	50.33	50.26	40.86	9.40	8.90	3.0
		50.19			8.40		
VI	Berkeley adobe	52.99	51.17	41.60	23.00	22.00	9.4
		49.35			21.00		
VII	Davis clay loam	36.19	37.45	30.44	15.72	15.72	7.7
		38.71			15.72		
VIII	Imperial Valley silty clay	36.54	37.03	30.10	2.55	2.80	1.8
		37.52			3.05		
IX	Manteca sandy	24.50	23.52	19.12	—.23	—.23	...
		22.54			—.23		
X	Fresno "white ash"	30.03	29.26	23.79	—.18	—.18	...
		28.49			—.18		
XI	Fresno sandy (Selma)	15.12	15.26	12.40	—.13	—.13	...
		15.40			—.13		
XII	San Diego (El Cajon) clay No. 2	40.67	41.72	33.91	0.95	0.75	0.4
		42.77			0.55		
XIIa	El Cajon sandy No. 4	38.64	39.20	31.87	—.33	—.33	...
		39.76			—.33		
XIII	Willows clay	35.07	33.46	27.20	0.65	0.68	0.3
		31.85			0.70		
XIV	Sacramento River alluvial	39.41	41.09	33.40	29.15	27.15	13.7
		42.77			25.15		
XV	Olinda sandy silt	35.21	34.65	28.17	—.18	—.22	...
		34.09			—.26		
XVI	Paradise Valley sandy silt	31.43	31.57	25.66	8.65	8.15	3.5
		31.71			7.65		
XVII	Napa Valley gravelly loam	46.41	46.90	38.13	7.80	.....	3.2
		47.39			....		
XVIII	Redlands clay loam (Grafton)	40.60	39.41	32.04	5.60	5.40	3.1
		38.22			5.20		
XIX	San Fernando sandy loam	54.74	53.27	43.30	14.40	15.40	7.5
		51.80			16.40		
XX	Eel River alluvial silt loam	44.17	44.17	35.91	29.00	27.00	6.5
		.....			25.00		
XXI	Salinas silt loam	28.28	29.96	24.35	31.65	30.15	16.4
		31.64			28.65		
XXII	Mattole River humus silt	25.48	27.51	22.36	0.70	0.65	0.1
		29.54			0.60		

ployed for the figures, we shall discuss the results obtained with every fertilizer separately before proceeding to the general appraisal of all the data and their significance.

Dried Blood

It appears very clear from the ammonification data in Table I that dried blood is very efficiently ammonified in nearly all of the soils tested. All but two of the twenty-three soils tested transformed, in 7 days under incubator conditions, more than 20 per cent of the blood nitrogen into ammonia and only one of the two exceptions was significantly below the 20 per cent standard. The best ammonia production was accomplished by the San Fernando sandy loam soil with a transformation record of 43.30 per cent based on the same criteria as the data above. Disregarding the Fresno sand, the total range of variation among the diversified group of soils used is less than 25 per cent in the total dried blood nitrogen ammonified. In other words, there appears to be a decided tendency toward a more or less uniform ammonifying efficiency among all the soils studied. To be sure the better soils do, in a general way, yield higher ammonification values than the poorer soils, but the differences obtaining between them are relatively small.

Comparing now the ammonification data with the nitrification data for dried blood nitrogen, we note first the striking dissimilarity between the direction and intensity of the two processes in any given soil. While it is true that in some soils the nitrification and ammonification processes are both vigorous, there appears to be no necessary correlation between them. Thus, while the San Fernando sandy loam accomplished the most vigorous ammonification of dried blood nitrogen among the soils tested, it is fifth on the list among the most efficient nitrifying soils. On the other hand, the Salinas silt loam which was among the weakest of the soils studied in ammonifying power, showed by far the highest nitrifying power both as regards the absolute amounts of nitrate produced and the percentage of the total nitrogen present transformed into nitrate. It appears, therefore, that a good ammonifying power of a soil is no criterion as to its nitrifying power. While it is true that most of the soils weakest in ammonifying power do not nitrify dried blood nitrogen, nevertheless, there are enough examples like the one above employed to leave no room for doubt as to the lack of correlation of the two processes in question.

High Grade Tankage

High grade tankage behaves very similarly to dried blood with respect to ammonification and nitrification in the soils under consideration here. With very few exceptions, tankage nitrogen does not become ammonified as readily in the soils studied as does dried blood nitrogen,

TABLE II

AMMONIFICATION AND NITRIFICATION OF HIGH GRADE TANKAGE

No.	Soil	Ammonification			Nitrification		
		Mg. N Over Blank	Average	% Made Available	Mg. N Nitrified	Average	% N Available
I	Anaheim	33.81	34.37	37.15	3.60	3.70	2.4
		34.93			3.80		
II	Oakley sandy	19.60	20.79	22.47	0.00	.....	...
		21.98			0.00		
III	Covina sandy	33.81	33.74	36.47	3.34	3.14	2.0
		33.67			2.94		
IV	Pomona sandy loam	37.52	38.08	41.16	25.45	25.45	11.4
		38.64			25.45		
V	Watsonville strawberry soil	37.31	36.82	39.81	5.50	5.70	2.1
		36.33			5.90		
VI	Berkeley adobe	37.03	37.87	40.94	12.30	12.70	6.2
		38.71			13.10		
VII	Davis clay loam	41.79	40.81	44.11	14.20	15.00	8.6
		39.83			15.80		
VIII	Imperial Valley silty clay	38.50	38.92	42.07	6.65	7.15	5.7
		39.34			7.65		
IX	Manteca sandy	21.70	21.98	23.76	—.05	—.08	...
		22.26			—.10		
X	Fresno "white ash"	27.51	26.18	28.30	0.05	0.05	Trace
		24.85			0.06		
XI	Fresno sandy (Selma)	15.26	14.56	15.74	0.00	.....	...
		13.86			0.00		
XII	San Diego (El Cajon) clay No. 2	34.37	35.14	37.98	2.50	2.40	1.8
		35.91			2.30		
XIIa	El Cajon sandy No. 4	31.36	32.13	34.73	3.75	3.25	2.6
		32.90			2.75		
XIII	Willows clay	28.35	28.21	30.49	2.10	1.95	1.3
		28.07			1.80		
XIV	Sacramento River alluvial	32.41	33.18	35.87	17.25	17.25	9.4
		33.95			17.25		
XV	Olinda sandy silt	33.81	34.79	37.61	0.85	0.80	0.3
		35.77			0.75		
XVI	Paradise Valley sandy silt	31.43	30.45	32.91	7.75	7.45	3.6
		29.47			7.15		
XVII	Napa Valley gravelly loam	37.03	37.73	40.78	7.90	7.90	3.7
		38.43			.....		
XVIII	Redlands clay loam (Grafton)	30.80	32.06	34.65	1.80	2.13	1.4
		33.32			2.45		
XIX	San Fernando sandy loam	40.18	39.69	42.90	20.50	20.50	11.7
		39.20			.....		
XX	Eel River alluvial silt loam	35.07	34.44	37.22	24.10	22.60	5.9
		33.81			21.10		
XXI	Salinas silt loam	29.12	29.68	32.08	30.75	31.75	20.7
		30.24			32.75		
XXII	Mattole River humus silt	31.64	30.52	32.99	1.30	1.35	0.4
		29.40			1.40		

but shows greater uniformity in that respect among them. Such inferior ammonifiability of tankage nitrogen as compared with dried blood nitrogen, is not reflected, however, in the nitrifiability thereof. On the contrary, more of the soils tested produced nitrates from tankage than from blood nitrogen and greater efficiency in that direction was attained by most of them as regards the percentage transformation of the total nitrogen into nitrate. Again, we find as we did in the case of dried blood that with a relatively weak ammonifying power the Salinas silt loam far surpasses in nitrifying efficiency any other soil in the series for tankage nitrogen. While in the soil in question the latter yields slightly less ammonia than blood nitrogen, it yields more nitrate nitrogen. On the other hand, the Davis clay loam which yields more ammonia from tankage nitrogen than any other soil of the series produces considerably less than half the amount of nitrate nitrogen produced by the Salinas silt loam. It must be added, too, that the San Fernando sandy loam which ammonified dried blood nitrogen most efficiently of all the soils tested, holds second place in that direction as well as in nitrifiability in the tankage series. We are confronted, therefore, by some cases of soils in which a good ammonifying power is accompanied by a good nitrifying power and by others, usually in the majority, in which no such relationship obtains. One more example may be cited from the tankage series to emphasize the point under consideration. The Imperial Valley silty clay and the San Fernando sandy loam show about equal ammonifying efficiency for tankage nitrogen, but the former transforms only about a quarter as much of the nitrogen present into nitrate as the latter. Based on ammonification standards alone the two soils appear to be equally good in rendering soil nitrogen available. Based on nitrification standards the first named soil cannot compare at all with the second.

Low Grade Tankage or Steamed Bone Meal

In Table III we see perhaps the most striking evidence of all concerning the point which we are endeavoring to elucidate. In this series the Olinda humus silt showing the highest ammonifying efficiency produced little or no nitrate, while the Anaheim sand with a very low ammonifying efficiency and near the minimum of the soils tested in that respect, stood third highest in nitrifying efficiency. On the other hand the Imperial Valley silty clay with a very high ammonifying efficiency for steamed bone meal nitrogen is also second in nitrifying efficiency. More clearly, therefore, than in any of the foregoing series the data of the steamed bone meal series fail to substantiate the common conception with regard to the necessary accompaniment of a good ammonifying power by a good nitrifying power. It is significant, however, that nitrification proceeds so efficiently in most of the soils when steamed bone

TABLE III

AMMONIFICATION AND NITRIFICATION OF LOW GRADE TANKAGE

No.	Soil	Ammonification			Nitrification		
		Mg. N Over Blank	Average	% Made Available	Mg. N Nitrified	Average	% N Available
I	Anaheim	3.15	3.72	12.27	13.80	13.30	14.3
		4.29			12.80		
II	Oakley sandy	11.78	11.14	36.76	6.88	6.88	11.4
		10.50			6.88		
III	Covina sandy	3.29	3.50	11.55	10.84	9.59	10.6
		3.71			8.34		
IV	Pomona sandy loam	12.88	12.11	39.96	18.37	18.37	11.4
		11.34			18.37		
V	Watsonville strawberry soil	9.73	9.80	32.34	5.00	5.00	2.5
		9.87			5.00		
VI	Berkeley adobe	11.13	11.48	37.88	10.10	10.10	7.2
		11.83			10.10		
VII	Davis clay loam	10.71	10.43	34.42	8.80	8.30	7.5
		10.15			7.80		
VIII	Imperial Valley silty clay	12.74	12.60	41.58	11.25	11.45	19.0
		12.46			11.65		
IX	Manteca sandy	11.48	12.04	39.73	2.85	2.55	3.6
		12.60			2.25		
X	Fresno "white ash"	10.71	10.64	35.11	2.40	1.90	3.1
		10.57			1.40		
XI	Fresno sandy (Selma)	8.96	9.24	30.49	—.70	—.73	...
		9.52			—.75		
XII	San Diego (El Cajon) clay No. 2	8.47	8.82	29.10	6.00	5.75	8.2
		9.17			5.50		
XIIa	El Cajon sandy No. 4	13.16	12.39	40.89	5.75	6.25	10.4
		11.62			6.75		
XIII	Willows clay	7.35	7.84	25.89	0.20	0.25	0.3
		8.33			0.30		
XIV	Sacramento River alluvial	8.33	8.33	27.49	15.25	14.25	11.8
		8.33			13.25		
XV	Olinda sandy silt	13.37	12.88	42.50	0.45	0.40	0.2
		12.39			0.30		
XVI	Paradise Valley sandy silt	12.53	12.81	42.27	6.75	7.05	5.0
		13.09			7.35		
XVII	Napa Valley gravelly loam	11.69	11.20	36.96	7.90	7.90	5.2
		10.71			.....		
XVIII	Redlands clay loam (Grafton)	3.92	3.50	11.55	7.30	7.60	9.5
		3.08			7.90		
XIX	San Fernando sandy loam	9.80	10.22	33.72	13.50	14.00	12.7
		10.64			14.50		
XX	Eel River alluvial silt loam	11.41	11.83	39.04	21.10	20.10	6.5
		12.25			19.10		
XXI	Salinas silt loam	7.56	7.35	24.25	19.75	18.75	20.8
		7.14			17.75		
XXII	Mattole River humus silt	10.92	11.48	37.88	1.00	1.10	0.3
		12.04			1.20		

meal nitrogen is supplied, and when a soil has a relatively feeble ammonifying power, quite as well as or better than when it has a strong ammonifying power. The possible causes underlying this unexpected state of affairs are discussed elsewhere (4).

Fish Guano

In general, the statements made with respect to high grade tankage are applicable in the case of fish guano. The nitrogen of the latter fertilizer is not as readily ammonified as that of the high grade tankage, but on the other hand, appears to be better suited to nitrification in most of the soils tested. A striking similarity between the amounts of ammonia produced from fish guano nitrogen by different soils is at once noticeable in a study of Table IV.

Eighteen out of twenty-three of the soils produce between 20 and 30 mg. of ammonia nitrogen in the stated incubation period, thus indicating a tendency already noted in the case of the other nitrogenous materials above discussed toward the obliteration of differences, if any exist, between different soils as regards powers of ammonification. This is clearly not so in the case of nitrification in respect to which we find marked discrepancies between different soils of wholly similar ammonifying power. Thus in the fish guano series the soil with the highest nitrifying power has only a mediocre ammonifying power, and *per contra* the soil with the highest ammonifying power has only a mediocre nitrifying power. Similar examples are quite common and could be cited from Table IV without difficulty. In common, therefore, with the other series above discussed the fish guano series does not appear to offer any evidence contradictory to the assumption above cited and which we are attempting to analyze here.

Cottonseed Meal

While cottonseed meal is more readily ammonified than steamed bone meal, it shows many resemblances to the latter form of nitrogenous fertilizer both in regard to ammonification and nitrification. Again, in the case of cottonseed meal nitrogen as in that of the other materials above described, the experimental data fail to establish any correlation between the relative availability thereof by the two methods under analysis. For example, the Salinas silt loam is No. 13 in point of efficiency as an ammonifier of cottonseed meal nitrogen, and yet is first in efficiency at nitrifying such nitrogen. On the other hand, the most efficient ammonifier of cottonseed meal nitrogen of the soils tested is the Napa gravelly loam, yet it is No. 16 in order of nitrifying power. These examples point clearly enough to the almost total lack of correlation between ammonifying and nitrifying efficiency of soils for a given kind of nitrogenous fertilizer.

TABLE IV

AMMONIFICATION AND NITRIFICATION OF FISH GUANO

No.	Soil	Ammonification			Nitrification		
		Mg. N Over Blank	Average	% Made Available	Mg. N Nitrified	Average	% N Available
I	Anaheim	22.05	22.40	26.44	3.80	4.00	2.7
		22.75			4.20		
II	Oakley sandy	17.36	16.80	19.83	0.13	0.10	Trace
		16.24			0.08		
III	Covina sandy	26.81	26.95	31.81	5.84	5.84	4.0
		27.09			5.84		
IV	Pomona sandy loam	28.42	27.23	32.14	19.37	19.37	9.0
		26.04			19.37		
V	Watsonville strawberry soil	26.67	26.53	31.32	11.90	11.70	4.5
		26.39			11.50		
VI	Berkeley adobe	27.23	28.07	33.14	12.30	11.70	6.0
		28.91			11.10		
VII	Davis clay loam	28.89	30.10	35.53	13.80	12.80	7.7
		30.31			11.80		
VIII	Imperial Valley silty clay	29.54	27.79	32.81	3.65	4.15	3.6
		26.04			4.65		
IX	Manteca sandy	17.22	17.01	20.08	0.05	0.09	Trace
		16.80			0.12		
X	Fresno "white ash"	23.31	23.03	27.19	0.20	0.18	Trace
		22.75			0.15		
XI	Fresno sandy (Selma)	12.60	12.18	14.38	—.05	—.05	...
		11.76			—.05		
XII	San Diego (El Cajon) clay No. 2	23.73	24.50	28.92	13.10	12.80	10.2
		25.27			12.50		
XIIa	El Cajon sandy No. 4	23.24	24.08	28.42	4.75	5.00	4.3
		24.92			5.25		
XIII	Willows clay	21.35	20.09	23.71	3.80	3.50	2.5
		18.83			3.20		
XIV	Sacramento River alluvial	25.83	24.99	29.50	16.25	16.75	9.5
		24.15			17.25		
XV	Olinda sandy silt	27.37	28.42	33.55	0.75	0.75	...
		29.47			0.75		
XVI	Paradise Valley sandy silt	30.57	30.73	36.24	8.35	8.05	4.1
		30.87			7.75		
XVII	Napa Valley gravelly loam	27.51	28.28	33.38	6.30	6.30	3.0
		29.05			.....		
XVIII	Redlands clay loam (Grafton)	20.30	20.79	24.52	8.90	8.35	6.1
		21.28			7.80		
XIX	San Fernando sandy loam	27.86	29.40	34.71	17.50	18.00	10.9
		30.94			18.50		
XX	Eel River alluvial silt loam	26.95	27.51	32.48	26.10	25.10	6.6
		28.07			24.10		
XXI	Salinas silt loam	24.64	24.85	29.33	25.75	25.75	17.7
		25.06			25.75		
XXII	Mattole River humus silt	24.78	23.94	28.26	1.00	1.10	0.3
		23.10			1.20		

TABLE V

AMMONIFICATION AND NITRIFICATION OF COTTONSEED MEAL

No.	Soil	Ammonification			Nitrification		
		Mg. N Over Blank	Average	% Made Available	Mg. N Nitrified	Average	% N Available
I	Anaheim	5.53	5.74	10.43	10.80	11.80	10.2
		5.95			12.80		
II	Oakley sandy	14.28	14.84	26.98	1.18	1.11	1.3
		15.40			1.03		
III	Covina sandy	10.43	10.50	19.09	14.84	14.84	12.9
		10.57			14.84		
IV	Pomona sandy loam	20.16	20.09	36.52	15.37	15.37	8.3
		20.02			15.37		
V	Watsonville strawberry soil	21.49	21.84	39.71	13.10	12.80	5.6
		22.19			12.50		
VI	Berkeley adobe	19.95	19.88	36.14	10.30	10.70	6.4
		19.81			11.10		
VII	Davis clay loam	19.67	19.88	36.14	7.80	7.80	5.7
		20.09			7.80		
VIII	Imperial Valley silty clay	21.84	21.56	39.20	11.65	11.15	13.1
		21.28			10.65		
IX	Manteca sandy	13.30	13.72	24.94	2.85	2.65	2.7
		14.14			2.45		
X	Fresno "white ash"	17.43	16.82	30.58	2.10	3.25	2.6
		16.31			2.40		
XI	Fresno sandy (Selma)	7.70	7.98	14.51	0.05	0.08	Trace
		8.26			0.10		
XII	San Diego (El Cajon) clay No. 2	17.85	17.92	32.58	11.50	11.10	11.6
		17.99			10.70		
XIII	El Cajon sandy No. 4	15.96	15.33	27.87	5.35	5.35	6.2
		14.70			5.35		
XIII	Willows clay	13.09	13.44	24.43	1.80	1.80	1.7
		13.79			1.80		
XIV	Sacramento River alluvial	14.63	14.56	26.47	14.25	16.25	11.2
		14.49			18.25		
XV	Olinda sandy silt	21.07	20.51	37.21	0.45	0.50	...
		19.95			0.55		
XVI	Paradise Valley sandy silt	20.65	20.30	36.91	6.75	6.65	4.0
		19.95			6.55		
XVII	Napa Valley gravelly loam	23.31	23.38	42.51	6.30	6.30	3.6
		23.45			.....		
XVIII	Redlands clay loam (Grafton)	11.90	11.20	20.36	13.30	12.80	12.1
		10.50			12.30		
XIX	San Fernando sandy loam	21.56	20.65	37.54	20.50	19.75	14.6
		19.74			19.00		
XX	Eel River alluvial silt loam	19.81	19.88	36.14	18.10	17.60	5.1
		19.95			17.10		
XXI	Salinas silt loam	16.94	16.73	30.41	23.75	24.25	21.0
		16.52			24.75		
XXII	Mattole River humus silt	14.00	14.91	27.10	1.20	1.20	0.3
		15.82			1.20		

General Discussion

Whatever attitude one may take with reference to the mode of procedure followed in these experiments so far as technique is concerned, there can be no doubt of the definite reply which our data give to the fundamental question herein involved. Questions as to the justice in such work of employing large quantities of a given fertilizer in making the tests would seem to have little or no cogency here since they would apply principally to those materials known as the "high grade ammoniates" like dried blood and high grade tankage. Such objections could not be valid in reference to cottonseed meal and certainly not to steamed bone meal. All forms of fertilizer nitrogen employed in our experiments have yielded one and the same significant result, namely, to point to the utter lack of dependability of ammonification data as indicators of the nitrification data that could be obtained with a given nitrogenous fertilizer. According to the ammonification data it is quite clear that dried blood takes the place of the most available form of nitrogen, tankage the second place, fish guano the third place, cottonseed meal the fourth place, and steamed bone meal the fifth place. On the other hand with nitrifiability as a criterion the order of importance of the materials named is almost exactly reversed.

There can be no doubt from the foregoing that the ammonifiability of a certain form of nitrogen in soil cultures is no dependable indicator of its nitrifiability. The practical question which now arises is as to whether this fact is of any significance. The answer to this question depends to a considerable degree on our interpretation of the data thus far gathered in different parts of the world on the proper form of nitrogen for plant nutrition. We venture the opinion based on a study of the data referred to that there is every indication at the present time that most plants prefer to subsist on the nitrate form of nitrogen. In stating this opinion, we are fully cognizant of the results of physiological experiments in solution cultures such as those of Hutchinson and Miller (2), and Schreiner (7), and others which showed that a number of plants appeared to be indifferent in their choice of a form of nitrogen. But we are constrained to attach more weight from the practical soil culture standpoint to the correlation, which has been drawn by Vogel (8) and others, between the nitrate supply in soils or their nitrifying power and the welfare of plants on such media. In our own experiments with plants in pot cultures, we have been able to establish much the same relative positions for the fertilizers in question, (with the inclusion of sulfate of ammonia), that are indicated in the tables here under consideration. We refrain from giving the pot culture data just referred to in this connection because they are to appear in another paper. Suffice it to say

here that we have accomplished the foregoing even when reasonable quantities of dried blood (e.g. 800 lbs. per acre) were employed on a sandy soil. The availability thereof as judged by the yields of barley obtained was far below that of sulfate of ammonia, cottonseed meal and steamed bone meal among other nitrogenous materials.

If, therefore, ammonification and nitrification data with fertilizers on different arid soils are not correlatable; if, further, nitrification data seem to be more reliable criteria on availability of nitrogenous fertilizers; and if vegetation experiments in soil cultures substantially confirm the indications of the nitrification tests, it would seem to be reasonable to employ the laboratory method for nitrification as a determinant of the relative availability of the nitrogen in different nitrogenous materials. This, of course, implies discarding the ammonification method. Whether the method of employing 1 per cent of the fertilizer in such nitrification tests should be modified to the use of much smaller quantities is, in this connection, merely a matter of detail which bears little cogency for the main question at issue, if our laboratory and vegetation tests may be taken as criteria.

We emphasize the relation of our results to arid soil conditions advisedly. We are not unaware of the good correlations drawn by J. G. Lipman, Brown and others between the ammonifying powers of soils and their crop producing powers; nevertheless, such correlations do not seem to be possible in the case of arid soils. The reasons for this discrepancy between the soil of the arid and of the humid regions are implied in our discussions in another paper (4). It should be observed further that with one and the same soil like the Oakley sand, the ammonification figures keep decreasing as one proceeds from the high grade organic nitrogenous fertilizers to the low grade fertilizers, while the opposite is true of the nitrification figures. Our greenhouse experiments are in accord with the nitrification figures in the Oakley sand.

As to the practicability of the nitrification method for the purpose in question, there can be little doubt. To be sure, the long incubation period required does make the method slightly cumbersome, but it must be remembered that many standard determinations in that direction, once made, will not have to be repeated. While more data are still to be obtained by us, we venture the suggestion tentatively that it may be possible to establish standards for certain groups of soils like those which are distinctly "arid" or distinctly "humid" which may serve as permanent standards. It must be added here that the data furnished in the tables submitted with this brief discussion bear primarily on arid soil conditions and may be the very reverse of those which may be obtained with truly humid soils by similar methods.

Conclusions

1. The ammonifiability data of fertilizer nitrogen in soils are not useful indicators of the nitrifiability data on the same fertilizers in the same soils.

2. In a group of soils, principally arid, tested with dried blood, high grade tankage, steamed bone meal, cottonseed meal and fish guano, the ammonifiability data gave the fertilizers in question the reverse position as to availability from those given them by the nitrifiability data.

3. Experiments in Europe and America appear to concede the paramount importance of the nitrate form of nitrogen for the nutrition of most plants. Hence the nitrifiability of a given form of nitrogen should be the most reliable laboratory criterion which we can employ.

4. The laboratory results are now being confirmed by vegetation experiments. Hence there appears to be no reason against adopting nitrifiability as the criterion of availability of nitrogenous fertilizers, for purposes of arid soil conditions at least.

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THE EFFECT OF PHOSPHORUS ON ALFALFA AND ALFALFA BACTERIA[1]

By

H. W. Truesdell[2]

Introduction

While it is generally agreed that, under average conditions, phosphorus treatment has a marked beneficial effect upon the growth of all plants, it has been observed that in the case of some legumes such as alfalfa this increase is especially great. Wing (24), who had great opportunity to observe field conditions, said, "Alfalfa especially revels in phosphorus, and, from our experience and observation on many farms, we know that it pays richly to go over unproductive meadows with an application of basic slag or acid phosphate. We apply about 400 pounds to the acre for top dressing. It is marvellous how this treatment with phosphorus will cause the alfalfa to spring into new life with such a vigor that it gets ahead of annual grasses and overshadows the weeds."

Recent investigators do not believe the benefit resulting from phosphorus treatment to be commensurate with the tissue requirements of the plant as shown by quantitative analyses. The benefit is far greater than such analyses would indicate. Fred and Hart (4, p. 36) and Lipman (11, p. 179) have considered a part of the benefit to higher plants from phosphorus treatment due to cellular stimulation and to the quickening of bacterial processes in the soil. In the case of legumes, however, there appears to be some additional factor which has not hitherto been considered.

It must not be supposed that the writer means that in all cases there is a greater absolute or percentage increase in the yield of legumes than in that of other crops. But, upon the basis of the relative phosphorus contents of the plant tissues, the increase seems to be greater in the case of the leguminous plants.

An examination of some plant analyses will serve to make this relation clearer. In the following figures certain legumes and non-legumes are compared in regard to their chief constituents.

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[1] Paper from the laboratory of agricultural bacteriology of the University of Wisconsin.

[2] The writer wishes to express appreciation for the help given him on this problem by Dr. E. B. Fred, in whose laboratory the work was carried on.

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PERCENTAGE COMPOSITION OF CERTAIN LEGUMES AND NON-LEGUMES
(N, P, K:20, p. 714-720) (Ca:1, p. 751) (S:6, p. 3)

	Nitrogen	Phosphorus	Potassium	Calcium	Sulfur
Alfalfa (*Medicago sativa*)	2.45	0.22	1.74	1.51	0.29
Red Clover (*Trifolium pratense*)..	2.10	0.22	1.65	1.43	0.16
Corn (*Zea mays*)	1.75	0.24	0.75	0.37	0.12
Timothy (*Phleum pratense*)	1.25	0.24	0.83	0.23	0.19

Since the process of nitrogen assimilation may be different in the two groups, legumes and non-legumes, it will not be considered. A comparison of the mineral elements in the two groups shows that potassium, calcium, and sulfur occur in much larger amounts in the legumes, while phosphorus occurs in about equal quantities in both groups. Exception must be taken in the case of the sulfur content of red clover and that of timothy. However, red clover is much lower than other legumes in sulfur content (6, p. 3). It is to be expected, therefore, that phosphorus fertilizers should have a proportionately slighter effect than potassium, calcium or sulfur fertilizers when applied to legumes as compared with similar treatments of non-legumes. Under average conditions no such relation has been reported. On the contrary, the evidence seems to indicate a disproportionate increase for phosphorus treatment in the case of legumes.

In attempting to explain the beneficial effect of phosphorus on legumes, a factor must be found which is not common to both legumes and non-legumes. Consequently, the possibilities of antagonism, neutralization of toxins, and stimulation of the common groups of soil bacteria may be dismissed as being in no way specific to legumes.

The stimulation of plants caused by phosphorus treatment is worthy of greater consideration. This stimulation must not be confused with simple nutrition, but rather must be associated with a nuclear stimulation resulting in increased cell division. While such stimulation obtains with all plants, it is possible that, in the case of legumes such as alfalfa, there may be a greater stimulation or that the plant, by means of its larger root system, is enabled to use this stimulation to greater advantage than are other plants. The writer noted an astonishingly strong growth of alfalfa plants fertilized with phosphorus. The beneficial effect of phosphorus was seen in very early stages before nodule formation had become very prominent.

There remains the possibility of the indirect increase in nitrogen nutrition of the leguminous host as a result of the increase in number of the nodule bacteria (*Bacillus radicicola*) in the soil, and consequent increase in numbers of nodules, as a result of more numerous infections and the greater proliferation of the bacteria within the nodules. This appears to be a highly probable explanation.

In order to understand the possibilities of stimulation of the lower plants, including the root-nodule bacteria, by phosphorus treatment, it may be well to give comparative figures showing the analyses of bacteria and legumes. Unfortunately no analyses are available for the nodule bacteria but it seems probable that they would be somewhat similar to *Azotobacter chroococcum*, a non-symbiotic nitrogen-fixing organism, for which figures are given.

PERCENTAGE COMPOSITION OF CERTAIN BACTERIA

(19, p. 495)

	Phosphorus	Potassium	Ash
Azotobacter chroococcum	2.23	2.00	8.20
Bacillus mycoides	1.77	1.88	7.50
Bacillus fluorescens liquefaciens	2.31	0.69	6.48

It will be noted from these figures that bacteria have a high ash content, a high potassium content, and an exceedingly high phosphorus content. The ratio of phosphorus to potassium, it will be noted, is very high.

PERCENTAGE COMPOSITION OF ROOTS AND NODULES FROM LUPINE

(*Lupinus luteus*) (18: p. 843)

	Total Ash
Nodules	6.32
Roots	4.55

ASH CONSTITUENTS

	Nodules	Roots
Silicon	1.59	1.90
Sulfur	4.90	6.38
Phosphorus	6.51	4.28
Potassium	17.31	12.05
Sodium	16.94	19.94
Magnesium	7.41	7.05
Calcium	7.64	12.04
Iron	0.83	0.75

It will be seen from the preceding figures that, in the case of lupine, the nodules are richer in ash, phosphorus, and potassium, than are the roots. It may be observed in passing that sulfur, an element essential to plant growth, which occurs in about the same quantities in soils as phosphorus, and which does not stimulate plants to any unusual growth, does not occur in as large quantities in the nodules as does phosphorus. By converting the percentages given above to a dry weight basis and comparing them with *Azotobacter chroococcum*, the following figures are obtained.

PERCENTAGE COMPOSITION OF LUPINE ROOTS AND NODULES, AND BACTERIA (19, p. 495; 18, p. 843)

	Phosphorus	Potassium	Ash
Lupine (*Lupinus luteus*) roots	0.19	0.54	4.55
Lupine (*Lupinus luteus*) nodules	0.34	1.09	6.30
Azotobacter chroococcum	2.23	2.00	8.20

From the preceding figures, it will be noted that the nodule analyses represent an approximate mean between the roots and bacteria in all three columns. Since the nodules are composed of bacterial cells and hypertrophied host tissues, this relation is what should be expected.

PERCENTAGE COMPOSITION OF ALFALFA AND BACTERIA

	Phosphorus	Potassium
Alfalfa (*Medicago sativa*) (20, p. 714)	0.22	1.74
Azotobacter chroococcum (19, p. 495)	2.23	2.00

It will be noted that, whereas there is not a great difference in the potassium content of alfalfa and bacteria, there is more than ten times as much phosphorus in the latter as in the former.

The foregoing figures indicate that phosphorus is the dominant element of the bacterial cell, and, from comparative compositions, phosphorus should cause a greater increase in growth of bacteria than in growth of higher plants. Consequently they seem to support the theory of the increase to the leguminous host as a result of increased activity of the nodule bacteria as outlined on page 78.

Review of Investigations on the Influence of Phosphorus on Bacterial Activity and Growth

While the increase in growth of the nodule organism, due to phosphorus treatment, has not heretofore received any direct attention, there are some interesting data, relating to bacterial stimulation and nodule formation caused by such treatment, which relate more or less closely to the subject.

The results of Lipman and Owen (13, p. 302) show that 1 per cent of acid phosphate causes a marked increase in the total number of bacteria in soil. Similar results were obtained by Fred and Hart (4, p. 54) as given in the following figures.

Treatment	Av. No. Bacteria in 1 gm. of Soil
None	3,082,000
Dipotassium phosphate, 500 mg.	5,920,000
Tricalcium phosphate, 1,000 mg.	3,176,000
Monocalcium phosphate, 1,000 mg.	4,230,000

Van Suchtelen (21, p. 77) found that the addition of superphosphate to soil double the evolution of carbon dioxide. Fred and Hart (4, p. 60) verified his results as shown by the following figures.

Treatment	Av. mg. CO_2 Evolution per Day from 100 gm. of Soil
None	12.75
Monocalcium phosphate, 1,000 mg.	19.28

Wohltmann, Fischer, and Schneider (26, p. 114) give us comparative figures for the effect of phosphorus on nitrification as shown below.

Treatment	Nitrification
None	100
Superphosphate	160

Considerable work has been done upon the effect of phosphorus on ammonification. The data of Wohltmann, Fisher, and Schneider (26, p. 108), Lipman (11, p. 179), Fred and Hart (4, p. 52), and McLean and Wilson (15) agree in showing a marked increase in ammonification for phosphorus treatment. The results of Lipman, which follow, show slighter benefit for phosphorus treatment than those of some other investigators.

Treatment	Ammonia Nitrogen found in 100 gm. of Soil mg.
None	133.7
Monocalcium phosphate	141.2
Dicalcium phosphate	142.0
Tricalcium phosphate	133.7

There have been several investigators working on the independent type of nitrogen fixation, especially that of *Azotobacter chroococcum*. Gerlach and Vogel (5, p. 638), working with solutions, made some interesting experiments concerning the effect of phosphorus. The results of these tests are given below.

Treatment	Nitrogen in 1000 c.c. of Solution mg.
Complete nutrient solution	28.7
Complete nutrient solution without calcium	4.6
Complete nutrient solution without potassium	24.1
Complete nutrient solution without phosphorus	3.7
Complete nutrient solution without phosphorus and potassium	1.8

It may be noted that the absence of calcium and phosphorus caused a great decrease in fixation and the absence of potassium a slight decrease. Wilfarth and Wimmer (22) secured similar results working in sand cultures. The latter likewise noted a similar relation of phosphorus to the growth and fixation of organic matter by algae. Working

in impure cultures of *Azotobacter*, Heinze (7, p. 904), and Hoffman and Hammer (8, p. 162), found a large increase in nitrogen fixed for mono-, di-, and tricalcium phosphate treatment. Koch, Litzendorff, Krull, and Alves (9, p. 413) working in soil got large increases for superphosphate added with dextrose as shown below.

Treatment	Nitrogen in 100 gm. of Soil mg.
None	46.4
Dextrose, 3 gm.	57.6
Dextrose, 3 gm., and superphosphate, 1 gm.	65.6

Their results show no increase for potassium sulfate and a decrease for potassium chloride treatment. Lipman (12, p. 139) found an increase of 50 per cent in nitrogen fixation for the addition of dipotassium phosphate in pure culture of *Azotobacter chroococcum*.

Of greatest interest from the standpoint of this paper are the data of many investigators on the effect of phosphorus treatment upon root nodule formation on the commoner legumes. Marchal (16, p. 1033) and Flamand (3) working in water cultures and Laurent (10, p. 1243), Wohltmann and Bergené (25), Löhnis (14, p. 26), Dehérain and Demoussy (2, p. 78), Prucha (17, p. 28), and Wilson (23) working in soil, all report a marked beneficial effect for phosphorus treatment upon nodule formation. Löhnis upon averaging a large number of plants obtained the following results with clover (*Trifolium pratense*).

Treatment	Nodules per Plant
None	31
Potassium phosphate	45

Wohltmann and Bergené, in an exceptionally comprehensive treatment of the subject, working with alfalfa treated with tripotassium phosphate, basic slag, and ammonium nitrate on many types of soils, got the following average results.

Treatment	Character of Nodules
None	Poor
Ammonium nitrate	Lacking
Basic slag	Good
Tripotassium phosphate	Very good

It will be seen from the preceding data that all investigations show an increased bacterial activity for phosphorus treatment.

Experimental Work

The vital question involved in this investigation is the discovery of a reason for the beneficial influence of phosphorus upon alfalfa and other legumes. As shown by chemical analyses, simple nutrition is insufficient

to explain this beneficial influence. This benefit cannot be explained by any factor which is common to both legumes and non-legumes.

The symbiotic nitrogen fixation of legumes does not occur in the common non-legumes. A likely explanation therefore, for the favorable influence of phosphorus in the former case is that it may cause greater growth and activity of the root bacteria, resulting in greater nitrogen fixation, and more rapid growth of the leguminous host. It is proposed to test the validity of this explanation experimentally.

One step favorable to the establishment of this theory has been made by the earlier investigators who observed the beneficial influence of phosphorus upon nodule formation. The effect of phosphorus upon the growth of the root bacteria and upon the nitrogen content of legumes has apparently not received careful study. The latter considerations will receive especial attention in this investigation.

The relation of phosphates to the growth of alfalfa, to the growth of alfalfa bacteria, or to the growth of a combination of these two factors furnishes the point of attack for the problem. The work naturally falls into two parts:—first, that which treats of the influence of phosphates upon the growth of the alfalfa organism (*B. radicicola*) as indicated by numerical counts; and second, that which treats of the influence of phosphates upon alfalfa, as regards nodule formation, rate of growth, dry weight of plants, and percentage and absolute content of nitrogen.

The Effect of Phosphorus in the Form of Various Phosphates on the Growth of Bacillus Radicicola from Alfalfa

In this laboratory work, sterilized soils, to which phosphates had been added, were inoculated with a pure culture of the alfalfa organism, allowed to incubate for one and two weeks, and the number of organisms in 1 gm. at the end of these periods determined. The results were compared with the results obtained from similar, inoculated, but untreated controls.

In selecting compounds for phosphorus treatment, it was decided to use some of the phosphates commonly found in soil and commercial fertilizers. Potassium, sodium, and calcium phosphates appeared to be the most suitable. Because of their reaction, the primary phosphates, which are acid, and the tertiary phosphates, which are alkaline, were rejected. In order that bacterial growth might be influenced as little as possible in this respect, the secondary phosphates, which are only slightly alkaline, were employed. The quantities of the phosphates expressed in the phosphorus equivalents 0.1, 0.02 and 0.002 per cent were thought to represent a suitable range of fertilizers.

The soil used in the experiment was neutral and presented a suitable medium for bacterial growth.

Effect of Dipotassium Phosphate on the Alfalfa Organism

In the first determination, dipostassium phosphate(K_2HPO_4) was used. This salt has the advantage, of having no harmful physical effect on soil and, on account of its solubility, of yielding its maximum benefits within a short period of time. It has the disadvantage of carrying much potassium, an important plant-food constituent, which may itself cause marked increase in growth.

Twenty-five-gram portions of Madison field soil (Miami silt loam) were placed in large test tubes. Dilutions of dipotassium phosphate were made, in such a way that 1-c.c. portions of the stock solution contained 0.125, 0.025, and 0.0025 gm. of the salt which, when added to the soil samples, was equivalent to 0.1, 0.02, and 0.002 per cent of elemental phosphorus. The salts were added as indicated in the plan below. A dilution of a pure culture of *B. radicicola* from alfalfa was made by transferring a loopful of culture from an agar slope to a sterile 100-c.c. water blank containing quartz sand and transferring a 5-c.c. portion to another 100-c.c. water blank. After sterilization for 4 hours at 15 pounds pressure, the soil tubes were inoculated, 5-c.c. portions from the highest dilution of the alfalfa organism being used. The water added with the inoculant brought the moisture content of the soil samples to about 28 per cent. The amount of the inoculation was determined by plate counts. The plan of the experiment is given below.

No. and Treatment	No. of Tubes in Each Group
1. Uninoculated	4
2. Inoculation	4
3. Inoculation with 0.002% phosphorus	4
4. Inoculation with 0.020% phosphorus	4
5. Inoculation with 0.100% phosphorus	4

Plate counts, to ascertain the number of bacteria in each soil sample, were made at the end of 7 and 14 days. In making the counts, duplicate tubes from each group were emptied into 400-c.c. water blanks, 25-c.c. portions from the latter transferred to other water blanks, and the pro-

TABLE I
THE EFFECT OF DIPOTASSIUM PHOSPHATE UPON THE GROWTH OF *B. RADICICOLA* FROM ALFALFA

Treatment in per cent of Phosphorus	Bacteria in 1 gm. of Dry Soil			
	After 7 Days		After 14 Days	
	Total	Increase Due to Treatment	Total	Increase Due to Treatment
None	24,400,000	.....	16,700,000	.....
0.002	28,500,000	4,100,000	18,100,000	1,400,000
0.02	158,000,000	133,600,000	113,100,000	96,400,000
0.1	58,500,000	34,100,000	40,300,000	23,600,000

cess repeated until five dilutions had been made. From the fourth and fifth dilutions plates were poured in triplicate, mannite agar being used. After five days of incubation at 30° C., the total number of colonies per plate was determined and the number of organisms in the original soil calculated. The results of the experiment are given in Table I.

It will be noted that there is an appreciable increase for the lowest dipotassium phosphate treatment, an enormous increase for the next treatment, and a somewhat smaller increase for the highest treatment. Obviously, the lowest treatment was insufficient to cause any large gain and the highest probably tended to have inhibitory as well as nutritive action, while the middle treatment approached more nearly the optimum concentration for the development of legume bacteria in this soil.

The objection may be raised that the results do not show the benefit for phosphorus alone since the potassium contained in the salt very probably caused a large part of the increased growth of the bacteria. In order to determine what the real effect of phosphorus may be in this case, the results must be compared with those obtained from the use of other phosphorus salts which do not contain potassium.

The Effect of Disodium Phosphate on the Alfalfa Organism

The experiment was next run with disodium phosphate ($Na_2HPO_4 \cdot 12H_2O$). This salt, it was thought, would not be open to the same criticism as dipotassium phosphate, inasmuch as sodium, unlike potassium is not usually considered to be essential to plant growth. On the other hand, unlike dicalcium phosphate, it is quite soluble. Its greatest defect is its tendency to deflocculate soil.

The process as outlined for dipotassium phosphate was repeated with disodium phosphate. In order to simplify the work, the highest phosphorus concentration was omitted. The stock solutions were made such that 2-c.c. portions contained 0.056 and 0.0056 gm. of the salt, and, when added to the 25-gm. samples of soil, were equivalent to 0.02 and 0.002 per cent of phosphorus. The results are shown in Table II.

TABLE II
THE EFFECT OF DISODIUM PHOSPHATE ON THE GROWTH OF *B. RADICICOLA* FROM ALFALFA

Treatment in per cent of Phosphorus	Bacteria in 1 gm. of Dry Soil			
	After 7 Days		After 14 Days	
	Total	Increase Due to Treatment	Total	Increase Due to Treatment
None	222,900,000	.....	260,600,000	.....
0.002	Lost	.....	481,100,000	220,500,000
0.02	392,800,000	169,900,000	534,500,000	273,900,000

Although sodium is supposedly a non-nutrient element, it may be seen from Table II that there is a very great numerical increase for the addi-

tion of disodium phosphate. This increase should, it would appear, be considered due to the phosphorus rather than to the sodium.

It will be seen that the counts were much higher in the case of disodium phosphate, although the percentage increase for treatment was much greater in the case of dipotassium phosphate. The difference may be due to a difference in moisture content or to a difference in soil. Unfortunately, the soil used for the dipotassium phosphate experiment was inadvertantly discarded during the summer, and the remaining tests had to be run on a different sample of soil, though of the same type.

However, with soils containing such an enormous number of bacteria as 500,000,000 per gram, comparison by percentage with soils containing 100,000,000 or less bacteria per gram, seems unjustifiable. In the former case, the bacteria are very likely approaching a point where they are self-inhibitory and thus cannot proliferate freely in response to an external stimulus, whereas, in the latter case, expansion is not limited.

Results similar to those shown in Table II were obtained by a subsequent determination. Flask cultures of the organism were treated with disodium phosphate and incubated as previously outlined. Counts were made in 7 days. The results which are represented by the sets of Petri dishes in Plate I, are given below.

Treatment in Per cent of Phosphorus	Bacteria in 1 gm. of Dry Soil
None	346,000,000
0.002	350,000,000
0.02	505,000,000

The Effect of Dicalcium Phosphate on the Alfalfa Organism

The final trial was made with dicalcium phosphate ($CaHPO_4 \cdot 2H_2O$). This salt is one of common occurrence in soil and a constituent of many phosphatic fertilizers. Although calcium is a recognized constituent of plant-food, it is generally believed to be present in the soil in sufficient quantities to supply the needs of plants, and it is not believed to cause any great increase in plant growth when applied in the form of its salts, unless it is applied in forms which may alter the reaction of the soil, or its physical character. It is likewise true that calcium salts are not reported to have any injurious effects upon plants or soil. However, the chief objection to the use of dicalcium phosphate was that it is very insoluble and, unless there is much carbon dioxide production, a large part of it is likely to remain unavailable within the limits of a short experiment.

The method as outlined for dipotassium phosphate was first employed. However, owing to the small increase in bacterial growth for the application of dicalcium phosphate, the relative variation was sufficient to render the results doubtful. It was, therefore, decided to repeat the experiment, using Erlenmeyer flasks. Portions of soil were weighed into each of ten 300-c.c. flasks, and treated as outlined for test tubes on page 84. For

each treatment duplicate flasks were used in place of the quadruplicate test tubes. In this case, 0.091 and 0.0091 gm. of the dicalcium phosphate, equivalent to 0.02 and 0.002 per cent of phosphorus, were weighed, and, on account of the insolubility of the salt, mixed directly with the soil in the flasks. The outline of the experiment is given below.

No. and Treatment	No. of Flasks
1. None	2
2. Inoculation	2
3. Inoculation plus 0.002% phosphorus	2
4. Inoculation plus 0.02% phosphorus	2

When counts were to be made, 25-gm. portions of soil from all of the flasks were weighed and transferred to water blanks. The flasks were then replugged, and returned to the incubator. After a week, other 25-gm. portions were weighed from the same flasks for the second count. In all other respects the experiment was similar to that in which dipotassium phosphate was used. This process called for more careful technique to prevent contamination, but gave more accurate results than did the test-tube method. The results are shown in Table III.

TABLE III

THE EFFECT OF DICALCIUM PHOSPHATE ON THE GROWTH OF *B. RADICICOLA* FROM ALFALFA

Treatment in per cent of Phosphorus	Bacteria in 1 gm. of Dry Soil			
	After 7 Days		After 14 Days	
	Total	Increase Due to Treatment	Total	Increase Due to Treatment
None	293,900,000	.....	227,200,000	.....
0.002	287,000,000	.....	233,800,000	6,600,000
0.02	367,400,000	73,500,000	260,500,000	33,300,000

On comparing Table III with the two preceding tables it will be observed that dicalcium phosphate produces nowhere near the increase in growth that dipotassium and disodium phosphates produce. This condition is to be expected from the insolubility of the salt.

The extreme difficulty in determining the effect of phosphorus in its calcium salts must be borne in mind. The secondary salt is insoluble, and the tertiary salt still more insoluble. The primary salt is soluble, and, from analogous results (*7*, p. 889) might cause greater increase than the secondary salt. However, the former has an acid reaction, and, since it is generally believed that acidity is unfavorable to the growth of the legume organism, the counts, if this salt were used, would still not be a measure of the beneficial action of phosphorus. Furthermore, in this experiment it seemed expedient to use the salt most comparable with the secondary sodium and potassium salts previously used.

With a due consideration for the character of the basic elements involved, it seems justifiable to conclude from the three preceding tables that phosphorus produces a decided increase in the growth of *B. radicicola* from alfalfa, analogous to that produced in the case of Azotobacter, and other soil forms.

The Effect of Phosphorus in the Form of Dicalcium Phosphate upon Alfalfa

The following experiment was carried on under greenhouse conditions using pot cultures. Alfalfa, grown on unsterilized soil, was inoculated, treated with phosphorus, and with phosphorus plus nitrogen. The results, in nodule formation, dry weight, percentage and absolute nitrogen content were compared with all possible control combinations.

The Experimental Method

Into 4-gallon earthen jars were weighed 14-kg. portions of neutral Madison field soil (Miami silt loam). The pots were treated as shown in the following outline.

Inoculated				Uninoculated	
Pot No.	Phosphorus as $CaHPO_4$ Per cent	Nitrogen as $CO(NH_2)_2$ Per cent	Pot No.	Phosphorus as $CaHPO_4$ Per cent	Nitrogen as $CO(NH_2)_2$ Per cent
1, 2	.....	.....	17, 18	.....	.....
3, 4	.....	0.014	19, 20	.....	0.014
5, 6	0.005	0.014	21, 22	0.005	0.014
7, 8	0.015	0.014	23, 24	0.015	0.014
9, 10	0.045	0.014	25, 26	0.045	0.014
11, 12	0.005	.....	27, 28	0.005	.....
13, 14	0.015	.....	29, 30	0.015	.....
15, 16	0.045	.....	31, 32	0.045	.....

The dibasic calcium phosphate was used because it is not likely to cause any great change in the reaction of soil to which it is added, and it should be expected to become soluble at a rate suitable to supply the needs of plants. The percentages of phosphorus, 0.005, 0.015, and 0.045, are equivalent to field applications of 700, 2100, and 6300 pounds of rock phosphate, the only comparable field fertilizer. As a nitrogenous fertilizer, urea [$CO(NH_2)_2$] was used because it was thought that it would not, either directly or indirectly by residues of decomposition, cause any marked change in the reaction of the soil, and because it has been shown that it does not injure nodule formation (3, p. 739). A quantity of urea equivalent to 0.014 per cent of nitrogen was used in order to furnish an excess of that element.

When the soil and fertilizer had been thoroughly mixed, the pots were ready for planting. Alfalfa seed was sterilized with a solution of mercuric chloride, washed in sterile water, and a portion of it soaked in a sus-

pension of alfalfa organisms. Pots 1 to 16 were planted with uninoculated seed and pots 17 to 32 with inoculated seed. In a week the seedlings were thinned to 30 plants to each pot.

Throughout the experiment the alfalfa was watered with distilled water in sterilized watering pots. The plants were grown for 7 months, during which four cuttings were made. When the experiment was discontinued, the roots were carefully removed, washed and the root nodules noted.

When the material had become thoroughly air-dry, it was weighed. In the case of the roots and of the first and third cuttings, the samples were ground and the percentage content of nitrogen determined by the Kjeldahl-Gunning method. The results, determined in percentage dry weight, were multiplied by the dry weights to give the absolute nitrogen content.

Early Effects of Phosphorus upon Alfalfa Seedlings

As previously stated (p. 78), it was observed in the earlier stages that there was a marked increase in growth for the addition of phosphorus. Nitrogen fertilization appeared to exert an injurious effect while inoculation seemed to have no effect whatever. The following figures will indicate the extent of this difference three weeks after planting.

Treatment—Inoculated and Uninoculated Series	Average Height in cm.
None	11.0
Nitrogen	7.0
0.005 per cent phosphorus with nitrogen	12.0
0.015 per cent phosphorus with nitrogen	14.0
0.045 per cent phosphorus with nitrogen	15.5
0.005 per cent phosphorus	15.0
0.015 per cent phosphorus	16.0
0.045 per cent phosphorus	16.5

The beneficial effect of phosphorus on plant growth was noted almost from the first, but, when the foregoing measurements were made, there was greater uniformity, permitting of more accurate measurement, than at any earlier period. At this early stage, the nodule bacteria could hardly be expected to have furnished the plant with sufficient nitrogen to cause such a difference in growth.

The rapid growth of the phosphorus-treated plants, it appears to the writer, may be accounted for only as a result of direct nutrition and stimulation of the plant by phosphorus, and as a result of the quickening of bacterial actions other than that of nitrogen fixation.

The Effect of Phosphorus on Alfalfa Nodule Formation

When the roots were removed the relative occurrence of the nodules was noted. Some nodules were found on all of the uninoculated plants although they were not so numerous as those on the inoculated plants. That the soil was already naturally inoculated with the alfalfa organisms

had previously been shown from the results of a preliminary test. However, it will be seen from the tables which follow, that there were perceptible differences resulting from inoculation notwithstanding the presence of the organism in the uninoculated controls.

On the roots of the phosphorus-treated plants there were more abundant nodules than upon those of the untreated controls. This is in agreement with the results of all previous investigators. Where both phosphorus and nitrogen were used, there was much variability but, on the whole, there were fewer nodules than where phosphorus alone was employed. Where nitrogen alone was used the results were similar to the controls.

The Effect of Phosphorus on the Air-dry Weight of Alfalfa

Although the air-dry weights of the alfalfa given in Tables IV and V fluctuate somewhat, the average of the duplicate checks, given in Table VI, brings out a definite relation between treatment and yield. Slight differences in the diameter of pots, chance inoculations, and attacks by insects were possible causes of the occasional disparity of duplicate checks.

TABLE IV

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT UPON THE AIR-DRY WEIGHTS OF ALFALFA: UNINOCULATED SERIES

Treatment		1st Cutting	2nd Cutting	3rd Cutting	4th Cutting	Roots	Total Roots and Tops
P as $CaHPO_4$	N as $CO(NH_2)_2$						
Per cent	Per cent	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
.....	.....	6.7	9.0	9.8	8.1	13.8	47.4
.....	.....	5.5	8.2	9.5	8.5	13.0	44.7
.....	0.014	5.7	10.1	12.2	10.4	18.8	57.2
.....	0.014	2.5	7.6	10.0	8.1	16.6	44.8
0.005	0.014	5.8	8.8	13.9	8.0	22.2	58.7
0.005	0.014	5.3	7.9	11.6	9.5	18.6	52.9
0.015	0.014	6.4	8.2	11.1	9.2	22.1	57.0
0.015	0.014	7.2	8.8	12.2	10.3	19.5	58.0
0.045	0.014	7.9	11.2	13.2	10.6	20 3	63.2
0.045	.....	6.0	8.5	9.7	9.8	17.0	51.0
0.005	.....	8.0	11.9	11.3	9.6	17.2	58.0
0.005	.....	6.7	9.8	10.0	9.0	17.3	52.8
0.015	.....	8.3	11.6	11.2	10.1	16.0	57.2
0.015	.....	7.9	10.5	11.7	10.9	16.9	57.9
0.045	.....	8.6	11.6	12.7	12.9	18.2	64.0
0.045	.....	9.8	10.4	10.9	10.7	16.7	58.5
Total		108.3	154.1	181.0	155.7	284.2	883.3

On comparing the vertical columns, it will be seen that the first cutting and the roots showed the greatest variation both between checks and between pots of unlike treatment, and that the third cutting was the most uniform. Nitrogen treatment had apparently a depressing effect in the first cutting, as previously noted, but had no harmful effects in subse-

TABLE V

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT UPON THE AIR-DRY WEIGHTS OF ALFALFA: INOCULATED SERIES

Treatment		1st Cutting	2nd Cutting	3rd Cutting	4th Cutting	Roots	Total Roots and Tops
P as $CaHPO_4$	N as $CO(NH_2)_2$						
Per cent	Per cent	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
.....	.....	6.0	9.8	11.0	10.9	16.7	54.4
.....	.....	5.0	10.4	9.8	8.8	13.6	47.4
.....	0.014	3.6	9.0	11.0	9.1	18.3	51.6
.....	0.014	3.0	7.6	11.8	9.8	15.0	47.2
0.005	0.014	7.8	12.3	14.0	9.7	18.2	62.0
0.005	0.014	4.0	8.8	13.0	9.9	16.2	50.9
0.015	0.014	6.2	11.1	14.0	12.0	21.3	64.6
0.015	0.014	6.8	9.6	12.7	11.5	17.5	58.1
0.045	0.014	6.5	10.2	12.2	11.6	18.0	58.5
0.045	0.014	7.5	8.5	13.4	9.9	16.2	55.5
0.005	.....	7.3	10.2	10.8	10.2	15.6	54.1
0.005	.....	6.4	8.5	12.4	9.9	16.0	53.2
0.015	.....	8.0	11.3	13.7	11.7	16.9	61.6
0.015	.....	7.0	8.8	14.2	10.4	17.2	57.6
0.045	.....	6.5	9.4	13.9	9.0	18.6	57.4
0.045	.....	6.6	8.7	12.7	10.5	17.8	56.3
Total		98.2	154.2	199.6	164.9	273.1	890.0

TABLE VI

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT UPON THE AIR-DRY WEIGHTS OF ALFALFA: AVERAGE OF DUPLICATES

UNINOCULATED

Treatment		1st Cutting	2nd Cutting	3rd Cutting	4th Cutting	Total Tops	Roots	Total Roots and Tops
P as $CaHPO_4$	N as $CO(NH_2)_2$							
Per cent	Per cent	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
.....	.....	6.10	8.60	9.65	8.30	32.7	13.40	46.1
.....	0.014	4.10	8.85	11.10	9.25	33.3	17.70	51.0
0.005	0.014	5.55	8.35	12.75	8.75	35.4	20.40	55.8
0.015	0.014	6.80	8.50	11.65	9.85	36.8	20.80	57.6
0.045	0.014	6.95	9.85	11.45	10.40	38.7	18.65	57.3
0.005	.....	7.35	10.85	10.65	9.30	38.2	17.25	55.4
0.015	.....	8.10	11.05	11.45	10.50	41.1	16.45	57.6
0.045	.....	9.30	11.00	11.80	11.80	43.8	17.45	61.3

INOCULATED

.....	.....	5.50	10.10	10.50	9.85	36.0	15.10	51.1
.....	0.014	3.30	8.30	11.40	9.45	32.5	16.65	49.1
0.005	0.014	5.90	10.55	13.00	9.80	39.3	17.20	56.5
0.015	0.014	6.50	10.35	13.45	11.75	42.1	19.40	61.5
0.045	0.014	7.00	9.35	12.80	10.75	39.9	17.10	57.0
0.005	0.014	6.85	9.35	11.60	10.05	37.9	15.80	53.7
0.015	.....	7.50	10.05	13.95	11.05	42.6	17.50	60.1
0.045	.....	6.55	9.05	13.30	9.75	38.7	18.20	56.9

quent cuttings. A matter of interest, for which no explanation will be given, is that those pots to which nitrogen had been added had very large root systems.

In a very general survey of the yields for the different treatments it may be noted that, upon comparison with the appropriate controls, there is a slight increase in growth for nitrogen, in the uninoculated, but not in the inoculated, a great increase for phosphorus and an equal increase for phosphorus plus nitrogen. A comparison of the uninoculated with the inoculated series indicates a trifling advantage for the latter.

The Effect of Phosphorus on the Air-dry Weight and Nitrogen Content of Alfalfa Roots

Table VII gives the dry weights, percentage of nitrogen, and the total nitrogen content of the alfalfa roots. Table VIII gives the averages of the duplicate jars. It will be noted that there is considerable variation in the percentage of nitrogen as well as in dry-weight.

TABLE VII

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE ROOTS OF ALFALFA

Treatment		Uninoculated			Inoculated		
P as $CaHPO_4$	N as $CO(NH_2)_2$	Dry Weight	Nitrogen	Total Nitrogen	Dry Weight	Nitrogen	Total Nitrogen
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.
.....	.....	13.8	2.74	378.1	16.7	2.78	454.3
.....	.....	13.0	2.60	338.0	13.5	2.83	382.1
.....	0.014	18.8	2.68	503.8	18.3	2.89	528.9
.....	0.014	16.6	3.01	500.0	15.0	2.81	421.5
0.005	0.014	22.2	2.56	568.3	18.2	2.82	513.2
0.005	0.014	18.6	2.71	504.1	16.2	2.97	481.1
0.015	0.014	22.1	2.66	587.9	21.3	2.83	602.8
0.015	0.014	19.5	2.54	495.3	17.5	2.80	490.0
0.045	0.014	20.3	2.56	519.7	18.0	2.82	507.6
0.045	0.014	17.0	2.64	448.8	16.2	2.72	440.6
0.005	.....	17.2	2.43	418.0	15.6	2.70	421.2
0.005	.....	17.3	2.51	434.2	16.0	2.65	424.0
0.015	.....	16.0	2.33	372.8	16.9	2.65	447.9
0.015	.....	16.9	2.35	397.2	17.2	2.96	509.1
0.045	.....	18.2	2.46	441.2	18.6	3.21	597.1
0.045	.....	16.7	2.60	434.2	17.8	2.80	498.4

The figures for the percentage of nitrogen appear to have little significance. It is apparent, however, that there is an increase in the total nitrogen content of the roots for the addition of phosphorus to the soil, and an even greater increase for the addition of nitrogen, and nitrogen with phosphorus. The total nitrogen content varies largely with the dry weights.

The Effect of Phosphorus on the Air-dry Weight and Nitrogen Content of the First Cutting of Alfalfa

In Table IX are shown the air-dry weights, the percentage of nitrogen and the total nitrogen for the first cutting. The averages for the duplicate jars are given in Table X.

From a comparison of these tables it will be seen that the percentage of

nitrogen, with a single exception, varies in inverse proportion with the dry-weights. This is in agreement with frequent observations that rapidly growing plants contain a smaller percentage of nitrogen, figured on the dry-weight basis, than slower growing plants. In this case, the phosphorus-treated plants grew much more rapidly than the controls, and the percentage of nitrogen is accordingly lower. It will, however, be noted that the total nitrogen content is greatest in the case of the phosphorus-treated jars.

TABLE VIII

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE ROOTS OF ALFALFA: AVERAGE OF DUPLICATES

Treatment		Uninoculated			Inoculated			Avg. Inoculated and Uninoc. Total Nitrogen
P as $CaHPO_4$	N as $CO(NH_2)_2$	Dry Weight	Nitrogen	Total Nitrog'n	Dry Weight	Nitrogen	Total Nitrog'n	
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.	Mg.
.....	.....	13.40	2.67	358.1	15.1	2.80	423.0	390.6
.....	0.014	17.70	2.85	501.9	16.7	2.85	475.2	488.6
0.005	0.014	20.40	2.64	536.2	17.2	2.90	497.2	516.7
0.015	0.014	20.80	2.60	541.6	19.4	2.82	546.8	544.2
0.045	0.014	18.65	2.60	484.3	17.1	2.77	474.1	479.2
0.005	.....	17.25	2.47	433.4	15.8	2.68	422.6	428.0
0.015	.....	16.45	2.34	385.0	17.1	3.81	478.5	431.8
0.045	.....	17.45	2.53	440.1	18.2	3.01	547.8	494.5
Average		17.80	2.59	460.1	17.1	2.83	483.3	.....

The Effect of Phosphorus on the Air-dry Weight and Nitrogen Content of the Third Cutting of Alfalfa

Neither the analyses for the roots nor those for the first cutting appear to the writer to be entirely satisfactory. That there is an increase in total nitrogen for phosphorus treatment is certainly evident, but the relation of the treatments to the percentage of nitrogen is not clearly shown.

The difficulty in the case of the roots is due to a number of conditions; first, it is difficult to remove all of the fine roots from the soil; second, it is difficult to free the roots from the small particles of soil; third, varying numbers of nodules are likely to be torn off of the roots, and fourth, the stubble retained on the roots may be quite variable. It will be readily seen that the loss of secondary roots will reduce the weight of the tissue, and the presence of dirt will increase the dry-weight and reduce the percentage of nitrogen. Likewise the quantity of the highly nitrogenous nodules and the amount of the comparatively, highly nitrogenous stem tissues retained may cause even greater variations in the nitrogen content of the roots.

In the case of the first cutting the difficulty, as previously mentioned, is the great influence of the rate of growth upon the nitrogen content. It is obvious that small differences due to treatment would, in this case, be entirely masked.

It seemed, therefore, advisable to take a later cutting, which showed a smaller difference in dry-weight indicating a more uniform growth. It was likewise thought that, at this later period, the normal metabolism of the mature plant would be more thoroughly established than at the time of the first cutting.

TABLE IX

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE FIRST CUTTING OF ALFALFA

Treatment		Uninoculated			Inoculated		
P as $CaHPO_4$	N as $CO(NH_2)_2$	Dry Weight	Nitrogen	Total Nitrogen	Dry Weight	Nitrogen	Total Nitrogen
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.
.....	.....	6.7	3.67	244.9	6.0	3.84	230.4
.....	.....	5.5	3.79	208.5	5.0	3.74	187.0
.....	0.014	5.7	4.33	246.8	3.6	4.77	171.7
.....	0.014	2.5	4.63	115.8	3.0	4.73	141.9
0.005	0.014	5.8	4.07	236.1	7.8	4.15	323.7
0.005	0.014	5.3	4.18	221.5	4.0	4.54	181.6
0.015	0.014	6.4	4.17	266.9	6.2	4.23	262.3
0.015	0.014	7.2	3.91	281.5	6.8	4.09	278.1
0.045	0.014	7.9	3.81	301.0	6.5	3.87	259.6
0.045	0.014	6.0	3.91	234.6	7.5	3.91	293.3
0.005	.....	8.0	3.80	304.0	7.3	3.68	268.6
0.005	.....	6.7	3.77	252.9	6.4	3.60	230.4
0.015	.....	8.3	3.69	306.3	8.0	3.61	288.8
0.015	.....	7.9	3.78	298.6	7.0	3.53	247.1
0.045	.....	8.6	3.78	325.1	6.5	3.42	222.3
0.045	.....	9.8	3.65	357.7	6.6	3.57	235.6

TABLE X

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE FIRST CUTTING OF ALFALFA: AVERAGE OF DUPLICATES

Treatment		Uninoculated			Inoculated			Avg. Inoculated and Uninoc. Total Nitrogen
P as $CaHPO_4$	N as $CO(NH_2)_2$	Dry Weight	Nitrogen	Total Nitrog'n	Dry Weight	Nitrogen	Total Nitrog'n	
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.	Mg.
.....	.....	6.10	3.73	226.7	5.50	3.79	208.7	217.70
.....	0.014	4.10	4.48	180.3	3.30	4.75	156.8	168.60
0.005	0.014	5.60	4.13	228.8	5.90	4.35	252.7	240.80
0.015	0.014	6.80	4.04	274.2	6.50	4.16	270.2	272.20
0.045	0.014	6.95	3.86	367.8	7.00	3.89	276.5	272.15
0.005	.....	7.35	3.79	278.5	6.85	3.64	249.5	264.00
0.015	.....	8.10	3.74	302.5	7.50	3.57	368.0	285.25
0.045	.....	9.20	3.72	341.4	6.55	3.50	229.0	285.20
Average		6.77	3.94	262.5	6.15	3.96	239.0	.....

Table XI shows the results for the third cutting and Table XII gives the averages for the duplicate treatments. This cutting was grown in the best of the season and as shown by Table VI, it had the maximum dry-

weight of all the cuttings. It represents a comparatively mature stage in the growth of the plant at a time when it was fairly free from insect and fungous attacks.

TABLE XI

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE THIRD CUTTING OF ALFALFA

Treatment		Uninoculated			Inoculated		
P as $CaHPO_4$	N as $CO(NH_2)_2$	Dry Weight	Nitrogen	Total Nitrogen	Dry Weight	Nitrogen	Total Nitrogen
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.
.....	.....	9.8	3.88	380.2	11.2	3.51	393.1
.....	.....	9.5	3.86	366.7	9.8	3.65	357.7
.....	0.014	12.2	3.60	434.2	11.0	3.49	383.9
.....	0.014	10.0	3.50	350.0	11.8	3.67	433.1
0.005	0.014	13.9	3.63	504.6	14.0	3.87	541.8
0.005	0.014	11.6	3.59	416.4	12.0	3.83	459.6
0.015	0.014	11.1	3.48	386.3	14.0	3.57	499.8
0.015	0.014	12.2	3.16	385.3	12.7	3.56	452.1
0.045	0.014	13.2	3.46	456.7	12.2	3.41	416.0
0.045	.....	9.7	3.49	330.5	13.4	3.92	525.3
0.005	.....	11.3	3.77	426.0	10.8	3.85	415.8
0.005	.....	10.0	3.99	399.0	12.4	3.50	434.0
0.015	.....	11.2	4.10	459.2	13.7	3.85	527.5
0.015	.....	11.7	4.09	478.5	14.2	3.79	538.2
0.045	.....	12.7	3.84	487.7	13.9	3.73	518.5
0.045	.....	10.9	3.95	430.6	12.7	Lost	Lost

TABLE XII

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE THIRD CUTTING OF ALFALFA: AVERAGE OF DUPLICATES

Treatment		Uninoculated			Inoculated			Avg. Inoculated and Uninoc. Total Nitrogen
P as $CaHPO_4$	N as $CO(NH_2)_2$	Dry Weight	Nitrogen	Total Nitrog'n	Dry Weight	Nitrogen	Total Nitrog'n	
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.	Mg.
.....	.....	9.65	3.87	373.5	10.50	3.58	378.2	375.4
.....	0.014	11.10	3.55	394.6	11.40	3.58	408.5	401.6
0.005	0.014	12.75	3.61	460.5	13.00	3.84	500.7	480.1
0.015	0.014	11.65	3.32	385.8	13.35	3.57	476.0	430.9
0.045	0.014	11.45	3.48	393.6	12.80	3.66	470.7	432.2
0.005	.....	10.65	3.88	412.5	11.60	3.68	424.9	418.7
0.015	.....	11.45	4.09	468.9	13.95	3.82	532.9	500.9
0.045	.....	11.80	3.90	459.2	13.30	3.73	518.5	488.9
Average		11.30	3.71	418.6	12.50	3.68	463.8	.....

The increase in total nitrogen and in dry weight of the tops due to phosphorus treatment is in entire agreement with the results for both the roots and the first cutting. But, *in this case, there is an increase in the percentage of nitrogen in the tops for phosphorus treatment.* It will likewise be noted that an abundant nitrogen treatment did not cause either as great a quantity or percentage of nitrogen to be stored in the tops as did the phosphorus treatment.

These results are quite clear cut and the data of the inoculated and uninoculated series agree throughout. Consequently this difference in the percentage of nitrogen must unquestionably be considered as resulting from phosphorus treatment.

The evidence points to a greater efficiency in fixing and storing nitrogen as well as an increase in growth as a result of phosphorus treatment. The nodule bacteria apparently have not only supplied more nitrogen to meet the needs of the larger, phosphorus-treated plants, but they have stored a larger percentage of nitrogen in the tops of these plants than in the untreated controls and the nitrogen-treated plants.

The data for the jar cultures of alfalfa seem to indicate an increased activity of the root bacteria due to phosphorus treatment, resulting in larger quantities of nitrogen being fixed and stored in the plant. In the case of the third cutting this relation is especially evident, since in the phosphorus-treated plants there are found to be not only greater quantities, but also a greater percentage of nitrogen than that occurring in the controls.

Summary and Conclusions

1. The relatively large increase in the growth of alfalfa and some other legumes resulting from phosphorus treatment, as compared with some non-legumes, seems to indicate some previously unaccounted-for benefit due to phosphorus treatment.

2. The relatively low phosphorus content of higher plants in comparison with relatively high phosphorus content of bacteria indicates that phosphorus may be expected to cause greater increases in the latter than in the former case.

3. That phosphorus does cause large increases in the growth and activity of various groups of bacteria, including some nitrogen-fixing forms is shown by the work of numerous investigators.

4. The work of some investigators has likewise shown that there are more numerous and larger nodules on the roots of leguminous plants as a result of phosphorus treatment.

5. The treatment of pure cultures of *B. radicicola* from alfalfa with phosphates resulted in large increases in the number of organisms varying with the character and solubility of the salt.

6. The fertilization of alfalfa plants with phosphate resulted in a much more rapid growth in the young seedling stage.

7. The fertilization of alfalfa with phosphorus resulted in increased nodule formation, dry weight, and total nitrogen content.

8. In the case of the third cutting, which was most nearly representative of normal, average conditions, not only was there an increase in the total nitrogen, but *there was an increase in the percentage of nitrogen associated with the addition of phosphorus fertilizer.*

9. The early increase noted in the growth of the young phosphorus-treated seedlings may be interpreted as a result of the nutrition of the plant and the stimulation frequently associated with cell reproduction and to the quickening of bacterial processes in the soil.

10. The ultimate increases in growth resulting from phosphorus treatment may, to a large extent, be due to the increased infection with the alfalfa organisms, increased growth and proliferation of the organism within the nodule, and consequently greater fixation of nitrogen. The increase in the percentage of nitrogen in the third cutting, resulting from phosphorus treatment, points strongly to this conclusion.

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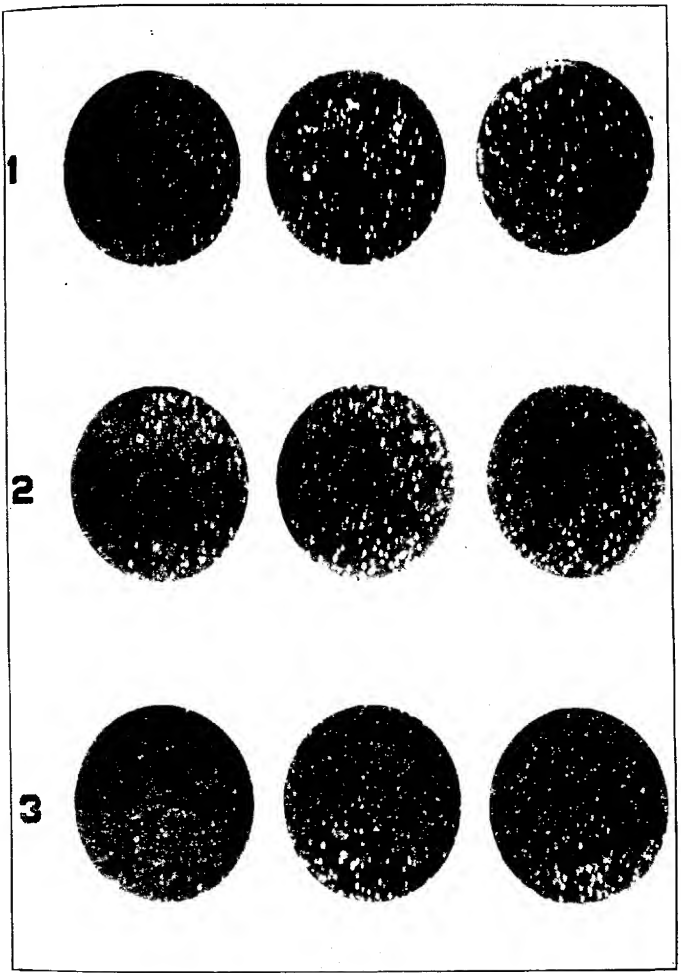
PLATE I

Colonies of *B. radicicola* from alfalfa in plate dilutions, showing the effect of phosphorus on the numerical increase of a pure culture of this organism in sterilized soil:

Fig. 1.—Control, representing 346,000,000 bacteria in 1 gm. of the original soil.

Fig. 2.—Disodium phosphate equivalent to 0.002 gm. of phosphorus added to the soil, representing 350,000,000 bacteria in 1 gm. of the original soil.

Fig. 3.—Disodium phosphate equivalent to 0.02 gm. of phosphorus added to the soil, representing 505,000,000 bacteria in 1 gm. of the original soil.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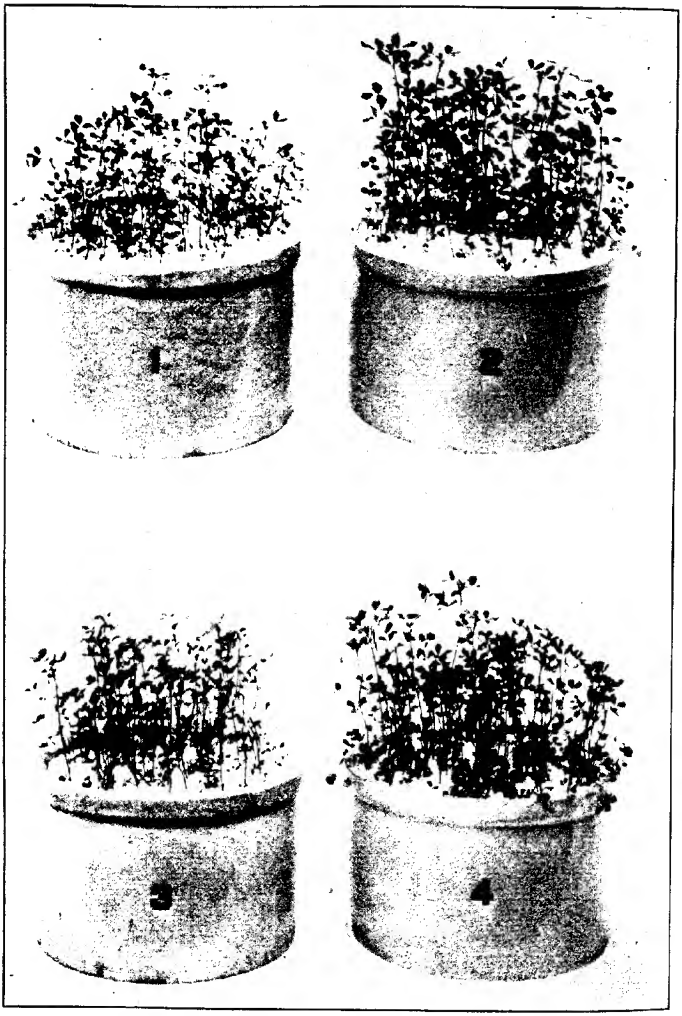
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PLATE II

Alfalfa plants, showing the effect of phosphorus upon the rate of growth.
Fig. 1.—Uninoculated; no phosphorus.
Fig. 2.—Uninoculated; dicalcium phosphate, 5 gm.
Fig. 3.—Inoculated; no phosphorus.
Fig. 4.—Inoculated; dicalcium phosphate, 5 gm.